IDRC-TS 7e

Nutritional Standards and Methods of Evaluation for Food Legume Breeders

Prepared by the International Working Group on Nutritional Standards and Methods of Evaluation for Food Legume Breeders

J.H. Hulse, K.O. Rachie, and L.W. Billingsley

Postal Address: Box 8500, Ottawa, Canada K1G 3H9
Head Office: 60 Queen Street, Ottawa

Hulse, J.H.
Rachie, K.O.
Billingsley, L.W.
IDRC IDRC-TS7e

Nutritional standards and methods of evaluation for food legume breeders. Ottawa, IDRC 1977. 100 p.

/IDRC pub CRDI/. Monograph on /nutrition/al /quality standard/s in /legume/ /plant breeding/ — discusses the /recommendation/s for standards of /food composition/; physical and /chemical analysis/, /food analysis/ and /biology/cal /evaluation technique/s.

UDC: 633.3 ISBN: 0-88936-137-1

Microfiche Edition $1

IDRC-TS7e

Nutritional Standards and Methods of Evaluation for Food Legume Breeders

Prepared by the International Working Group on Nutritional Standards and Methods of Evaluation for Food Legume Breeders

J.H. Hulse,* K.O. Rachie,* and L.W. Billingsley**

This publication was prepared by the International Development Research Centre in cooperation with the International Union of Food Science and Technology, the International Union of Nutrition Sciences, the International Centre of Tropical Agriculture (CIAT), the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), and the International Centre for Agricultural Research in Dry Areas (ICARDA).

*Cochairmen of the Working Group. **Editor.

Cochairmen

Hulse, J. H. Director, Agriculture, Food and Nutrition Sciences, International Development Research Centre, Box 8500, Ottawa, Canada

Rachie, K. O. Associate Director General Research, CIAT, Apartado Aéreo 67-13, Cali, Colombia

Participants

Bender, Arnold University of London, 59 Perryn Road, London W3 7LS, England

Bressani, Ricardo Head, Division of Agricultural and Food Sciences, INCAP, Apartado Postal No. 1188, Guatemala City, Guatemala

Carpenter, K. J. Department of Nutritional Sciences, University of California, Berkeley, California 94720, USA

Dovlo, Florence Head, Food Research Institute, P.O. Box M20, Accra, Ghana

Green, J. M. ICRISAT, 1-11-256 Begumpet, Hyderabad 500016, India

Hawtin, Geoffrey ALAD, The Ford Foundation, P.O. Box 2344, Cairo, Egypt

McLaughlan, J. M. Food Directorate, Health Protection Branch, Health and Welfare Canada, Tunney's Pasture, Ottawa, Canada

Tkachuk, R. Grain Research Laboratory, Canadian Grain Commission, 303 Main Street, Winnipeg, Manitoba, Canada

Williams, P. C. Grain Research Laboratory, Canadian Grain Commission, 303 Main Street, Winnipeg, Manitoba, Canada

Rapporteur

Billingsley, L. W. Ottawa, Canada

Contents

Chapter 5. Background Papers

Foreword

The complementary role in human nutrition of the proteins of cereals and food legumes is a well-established fact. Nonetheless, compared with work on the major cereals, little effort has been devoted by agricultural scientists to legume improvement (if the soybean is excluded). World average yields of the main legumes (excluding soybeans) are of the order of 0.5 ton per hectare, compared with ca. 2.8, 2.25, and 1.7 tons per hectare for maize, rice, and wheat, respectively, and nothing comparable to the prolamin-depressant or "high-lysine" genes found in maize and barley has been reported among the legumes.

In recent years, following the remarkable success of the International Maize and Wheat Improvement Centre (CIMMYT) and the International Rice Research Institute (IRRI) in breeding higher-yielding varieties of wheat and rice, other international agricultural research centres (IARCs) have come into being under the auspices of the Consultative Group on International Agricultural Research (CGIAR). Several of these centres are dedicated to the improvement of various important legumes. For example, the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) includes in its program work on chick-pea and pigeon pea, the International Institute of Tropical Agriculture (IITA) on cowpeas, and the International Centre for Tropical Agriculture (CIAT) on field beans. The International Centre for Agricultural Research in Dry Areas (ICARDA), a new centre in the Middle East, will be responsible for faba bean and lentil improvement.

In May 1974, the United Nations Protein Advisory Group (UNPAG) and the International Development Research Centre (IDRC) jointly sponsored a Working Group that met at CIMMYT and prepared the PAG Guideline (No. 16) on Protein Methods for Cereal Breeders as Related to Human Nutrition Requirements. The guideline has also been published by the American Association of Cereal Chemists in *Advances in Cereal Science and Technology*.

In January 1975, at a meeting of legume breeders held at ICRISAT in India, the need was expressed for a similar guideline for legume breeders. Following later discussions with representatives of the IARCs and the president of the International Union of Food Science and Technology (IUFoST), who contacted the International Union of Pure and Applied Chemistry (IUPAC) and the International Union of Nutrition Sciences (IUNS), the IDRC undertook to sponsor jointly with the international bodies named, a working group to propose standards of nutritional quality to be aimed for by legume breeders, and to prescribe the methods of physical and chemical analysis and biological evaluation relevant to the proposed standards.

The working group, representative of the sponsoring organizations and including experts from plant breeding, analytical chemistry, nutritional biochemistry, and other relevant disciplines, convened at the headquarters of IDRC in Ottawa from 5 to 7 July 1976.

The text that follows represents the recommendations of the working group. It is hoped that what is proposed will be of interest and assistance to legume breeders, analysts, nutritionists, and all others directly concerned with the improvement and wider use of food legumes in human diets. The group recognizes that studies proceeding in many centres may lead to improvements in the methodology outlined here. As better methods become validated, they will have to be included in a revision. However, the adoption at present of some standardization will mean that results reported from widely separated research centres can be compared with confidence.

Many of the recommendations and the supporting text are derived from research papers and other scientific publications. Reprints of the original papers cited in the references are available to scientists in developing countries upon request to the International Grain Legume Information Centre, at IITA, Barclays Bank Chambers, Bank Road, P.M.B. 5320, Ibadan, Nigeria.

J.H. Hulse
Director
Agriculture, Food and Nutrition Sciences
International Development Research Centre

Chapter 1

Introduction

The importance of the food legumes, particularly in the diets of undernourished peoples, has been emphasized by several international bodies. The Protein Calorie Advisory Group of the United Nations stated:

> "The food legumes are important and economical sources of protein and calories as well as of certain vitamins and minerals essential to human nutrition. However, the significant role they play in the diets of many developing countries appears to be limited by their scarcity, caused in great part by their present low yield, their consequent cost, and certain defects in their nutritional and food use qualities."

The food legumes include those species of the Leguminosae that are used as human food as distinct from oil-bearing legumes and pasture or forage legumes. Food legumes are eaten as mature dry seeds, in which form they are usually known as "pulses," or as green seed pods in which the immature seeds are contained.

Increasing and stabilizing yields should be the first and immediate goal of the major pulse improvement program, and it is expected that the emphasis will continue to favour an improved capacity to produce dry seeds as a means of increasing the indigenous food supply and generating farmer income.

It is also essential to increase the food use acceptability and nutritional values of the pulses as well as to reduce the risk of losing desirable quality factors in their genetic improvement. This publication identifies and defines food use and nutritionally related problems inherent in pulses. It also defines specific principles and guidelines in quality improvement, and provides detailed methods for evaluating and screening pulse crops in breeding programs. International pulse improvement programs can include these vitally important food quality improvement objectives concurrently with those of an agronomic/production nature.

The proteins of cereal grains and food legumes supplement each other nutritionally because each is comparatively rich in the amino acids in which the other is deficient. The optimum combination is provided by a diet in which roughly half the protein requirement is provided by cereals and half by legumes.

In spite of their nutritional importance as a source of food protein, food legumes have until recently received much less attention by plant breeders than the cereal grains. This results from the fact that pulses are less likely to provide a satisfactory economic return to farmers who grow them. High-yielding varieties of wheat and rice are more economically attractive than food legumes to Asian farmers; this is verified by the comparative increase in cereal production, and decline in food legume production to their present ratio of roughly 9:1.

During May 1974, UNPAG, together with CIMMYT, IUNS, and IDRC, jointly sponsored a Working Group that met at CIMMYT in Mexico and prepared PAG Guideline No. 16, *Protein Methods for Cereal Breeders*. The document prescribed specific physical and chemical methods to determine protein quality and quantity suitable for use in field laboratories, as well as in

central laboratories serving cereal breeding improvement programs. Early in 1976 an informal working group of IUFoST urged that a working group be formed to provide a manual of recommended methods to evaluate the nutritional and functional properties of pulses. In response to this recommendation, a working group met at the headquarters of IDRC in Ottawa, Canada, during July 1976. Within the membership, the following organizations were represented: IUFoST, IUNS, CIAT, ICRISAT, ALAD/ICARDA, and IDRC. The members of the group were: J.H. Hulse and K.O. Rachie (Chairmen), A.E. Bender, R. Bressani, K.J. Carpenter, F.E. Dovlo, J.M. Green, G.C. Hawtin, J.M. McLaughlan, R. Tkachuk, P.C. Williams, and L.W. Billingsley (rapporteur).

The contents of this publication represent the opinions of the group relevant to the subject under discussion. The text was circulated to the organizations represented by the working group members for ratification.

The legumes considered by the working group are those included in the programs of the IARCs, namely:

Phaseolineae
- Dry beans (*Phaseolus vulgaris*)
- Cowpeas (*Vigna unguiculata*)
- Pigeon peas (*Cajanus cajan*)
- Mung beans (*Vigna radiata* var. *aureas* and *mungo*)

Vicieae
- Chick-peas (*Cicer arietinum*)
- Broad beans (*Vicia faba*)
- Lentils (*Lens culinaris*)
- Dry peas (*Pisum sativum*)

The present publication includes: (1) A discussion of the nutritional objectives to which legume breeders should give attention; (2) recommended physical and chemical methods of analysis; (3) recommended methods of biological evaluation; and (4) a series of related background papers written by various members of the working group.

Purpose and Objectives

The special contribution of food legumes to the human diet lies in the quantity and quality of their protein content. The group therefore concerned itself with the nutritional factors to be assigned highest priority in legume breeding programs. It also considered methods for monitoring the results of breeding programs in terms of true nutritional value.

The characteristic of improved breeding material that has first priority in food legume and other programs is that of high yield. A cultivar is unlikely to be adopted by farmers unless it outyields other cultivars. Consumer acceptance of a new line is also essential. The price, appearance, cooking qualities, and flavour will all influence consumers, particularly those with low incomes who are most likely to suffer from undernutrition or malnutrition.

The acceptability of food legumes varies widely both among and even within communities, and acceptability tests cannot be readily carried out at an IARC. It is necessary, particularly within national breeding, adaptation and selection prorams, to collect as much information as possible about the characteristics that influence the principal consumers of food legumes and pulses, particularly those in developing countries. An IDRC publication covers this subject in part (1).

A special problem with some food legumes is that they require long cooking, with high consumption of fuel. This is an important obstacle to use by low-income groups, and new cultivars should have relatively short cooking times. Certain standardized tests are therefore proposed for the assessment of this characteristic.

The objectives of prime importance in legume breeding programs include the following.

Nutrient content

Legume breeders seek first to synthesize and identify cultivars capable of high and stable yields (i.e., weight of mature seed per unit area of land per unit of time) and wide adaptability to a range of agroenvironmental conditions. However, selection for higher yields should not be prejudicial to the content of nutritional quality of the legume protein. Therefore, one of the principal breeding objectives should be to increase the total quantity of digestible protein produced per unit area of land per year. This can be achieved by increasing the average protein–nitrogen content in terms of nitrogen per seed and protein as a percentage of total edible dry matter, concomitant with yield.

In cassava-based diets, legumes provide protein in which the methionine and cystine content are important. In diets in which cereals provide the main source of calories, there is less danger of protein deficiency if the amounts of food eaten are sufficient to meet energy requirements. But in cereal/pulse diets, the high lysine content of the legumes is important in compensating for the deficiency of lysine in cereal grain proteins. Consequently, the levels of these amino acids in new cultivars should not be allowed to decrease below levels presently available.

Digestibility

The digestibility of both protein and carbohydrate fractions in a number of food legumes is significantly lower than that of the carbohydrate and protein fractions of cereal grains. Pulses of higher digestibility would increase the "effective" supply of nutrients even if the amount eaten (intake) remained unchanged.

Adverse factors

Certain legumes are inclined to induce flatulence (the production of intestinal gases). Flatulence is believed to be caused by indigestible sugars (stachyose, raffinose), which are fermented by intestinal bacteria with production of gas that leads to discomfort and sometimes pain, particularly in infants.

Many other adverse or antinutritional factors in food legumes are known (see appended paper), most of which are almost completely destroyed during normal cooking. It is necessary to check the extent of destruction during cooking, before the release of a new cultivar (2).

Certain polyphenols, as yet unidentified, may be in part responsible for the low digestibility of some legume species. The group considered it essential to identify and classify the pigments present in highly coloured legume seed coats, to determine what, if any, effect they have on nutritional quality, and what is their effective function in the plant.

Genetic Improvement

The most important pulses include four species in each of the families Phaseoleae and Vicieae, as listed in Table 1. This table gives some indication of

the magnitude of initial germ plasm screening, approximate current production, estimated numbers of accessions available, and predictions of the size of these collections by the end of the present decade (1986). It also illustrates the present importance, wide acceptance, and use of these crops throughout the developing regions of the world.

Some of the difficulties encountered by plant breeders are due to the magnitude of the germ plasm collections and the numbers of cultivars produced by crossing parental materials in breeding programs, which produces large numbers of seed samples. However, the size of these samples, particularly in early generations of breeding materials, may be very small. In the international legume breeding program conducted in the IARCs, two generations may be grown in a single year with only a short time between the first harvest and the second planting. Consequently, the breeder needs screening methods that are rapid, economical of material (ideally nondestructive), and sufficiently precise and reproducible to be generally applicable, so that different research centres can adopt standard procedures that will yield comparable results. A more detailed account of a typical breeding procedure appears in the first of the appended papers.

The utilization and broad adaptation of the eight species listed in Table 1 are briefly described as follows:

Dry beans — for intermediate elevation tropics (800–2400 m) and for drier, moderate temperature, irrigated conditions.

Pigeon peas — for the humid to semi-arid low to intermediate elevation tropics.

Cowpeas — for the semi-arid to subhumid lowland tropics.

Mung beans — for the subhumid lowland tropics and subtropics.

Chick-peas — for cooler environments including the higher elevation tropics and the Mediterranean winter–spring seasons.

Broad or Faba beans — for cooler environments including higher elevations and Mediterranean winters.

Lentils — for semi-arid higher elevations and Mediterranean winter - early spring seasons.

Peas — for intermediate to high elevation tropics, and as a winter crop in Mediterranean-type environments.

National and international collaboration

The pulse improvement programs of the IARCs were established primarily to serve national breeding programs through: (1) assembling, classifying, maintaining, and distributing germ plasm; (2) developing and supplying breeding populations with sufficient diversity to be used in different environments; (3) coordinating international trials to facilitate multilocation testing; (4) conducting workshops for breeders and providing training; (5) maintaining a close liaison with national programs through personal visits and distributing newsletters, reports, and other information; and (6) strengthening cooperative programs by providing staff and support.

It is necessary to maintain genetic diversity for location specificity for yield, disease resistance, agronomic requirements, and visible quality factors (such as seed size, colour, and texture), in improved populations developed at the IARCs. On the other hand, higher protein or sulfur amino acids, digestibility, and biological value could be selected initially at one location.

Table 1. Status of inclusive germ plasm collections of important pulses.

Common name	Scientific name	Responsibility	Approx. production (metric tonnes)		Approx. no. of accessions	
			World	Tropics	1976	1986 (est.)
Phaseoleae						
Dry beans	*Phaseolus* species[a]	CIAT	11.7	5.5	13000	28000
Cowpeas	*Vigna unguiculata*	IITA	2.0	1.7	9000	20000
Pigeon peas	*Cajanus cajan*	ICRISAT	2.3	2.3	7000	16000
Mung beans	*Vigna radiata* var. *aureus*	AVRDC[b]	1.5	1.3	3000	9000
Vicieae						
Chick-peas	*Cicer arietinum*	ICRISAT	6.7	5.7	10600	20000
Broad beans	*Vicia faba*	ICARDA	5.2	0.5	1000	4000
Lentils	*Lens culinaris*	ICARDA	1.1	0.5	2500	6000
Dry peas	*Pisum sativum*	—	11.0	1.2	—	—

[a]Includes primarily *P. vulgaris* and *P. lunatus*, also some *P. coccineus* and *P. acontifolius*.
[b]Asian Vegetable Research and Development Center, Shinhua, Taiwan.

Staged screening procedures

The concept of the plant-improving process can be seen as a sequence of stages with increasing precision and accuracy. The progression may be complicated by many materials that must be processed within a short period of time, in the event of growing two or more sequential breeding generations in 1 year. It is generally agreed that the first step in selecting improved parents for hybridization requires the screening of inclusive germ plasm collections for desired characters. As an initial guide to the magnitude of this preliminary screening operation, Table 1 indicates the size of existing collections and provides an estimate of the expected numbers of those accessions that will accumulate by 1986. In most cases 10–25-g samples of seed of each entry could be made available for the initial screening process. Larger amounts can usually be provided when it is necessary to confirm preliminary observations.

Modern genetic principles recognize the importance of simply inherited characters controlled by one or a few genes, and of more complex characters controlled by many genes and gene interactions. These two kinds of genetic mechanisms dictate the breeding procedures and strategies to be followed. Simply inherited characters may be closely linked or associated with certain easily recognized morphological characters. This possibility, though likely to be low in frequency, should be recognized as its occurrence significantly increases the rate of improvement.

There is relatively little information available on the inheritability of quality factors in the pulses, so that initial screening of the many entries in the basic germ plasm collections is of primary importance. The second step involves intercrossing the extremes of these types and ascertaining through genetic procedures the inheritability of the characters. The three major screening quality evaluation stages are as follows:

1. Quick screening methods — These are required for the basic germ plasm and early segregating materials in the F_2 and F_3 generations. Numbers ranging from 5000 to 15 000 lines may have to be screened in basic germ plasm collections, and at least 10 000 – 12 000 plant progenies of early generation materials from F_2 and F_3 plants may need to be screened each growing season in major pulse improvement programs at the IARCs. It is estimated that 10–25 g of seed (depending on the species) would be made available from the basic germ

plasm collections and that from 10 to 100 g of seed could be available from plant progenies in early generations of the breeding program, as shown in Table 2.

2. Verification of the late lines — The second stage involves retesting of a selected few lines representing about 2–10% of the basic materials that have also been grown at a range of locations to ascertain the environmental stability of the quality characters involved. For this purpose, large-scale precise techniques are required, as it is necessary to evaluate up to several hundred such lines. Initially, up to 1000 selected parental lines might be evaluated for each desired character, whereas in the later generations, perhaps as many as 300 advanced lines from the F_5 generation onward would require this assessment. Considerably more material could be provided at this stage, i.e., from 500 to 2000 g (Table 2).

3. Final biological evaluation — The most elite genetic stocks or parental lines and advanced materials from the F_5 generation onward should be given biological evaluation utilizing small animals. The numbers of such advanced lines are estimated at 15 to about 40 each season, with from 1000 to 2000 g being made available for the assay. Simultaneous evaluation should be made of the complete amino acid profile, cooking quality, and consumer acceptability. Chemical and statistical analysis on these advanced materials will indicate where genotype by environmental interactions occurs. The breeder would also include in his description of advanced materials being distributed the dehulling percentage, if important, and gross quality characters of the seeds such as coat colour, shape, size, and texture.

The breeding process

There are several important steps in the genetic improvement of product quality in pulses in conventional breeding procedures. Beginning with the basic germ plasm, these include:

(1) Preliminary screening of inclusive germ plasm collections for desired characters;
(2) Identification of elite parental stocks as progenitors in the improvement process;
(3) Determination of the degree of genetic control of the characters involved;
(4) Intercrossing of the elite progenitors in various combinations depending on overall objectives and the breeding strategy determined from examining genetic control;

Table 2. Stage and amount of material for seasonal selection of early generation lines in large-scale breeding programs.

Stage/generation	Samples per crop stage	Seed quantity per sample, g[a]
Germ plasm collection	5000–15000[b]	10–25
Selected parental lines	1000	500
F_3 seeds (F_2 plants)	1000	10–100
F_4 seeds (F_3 plants)	10000	10–100
Advanced lines (F_5)	300	1000–2000

[a]Wide variation in seed quantities reflects the range in seed size among edible pulses (30–2000 mg/seed).
[b]Repeated testing of germ plasm not essential; these numbers would be greatly reduced after initial stages of the program.

(5) Early generation grow-outs — F_1 plants carrying F_2 seeds are usually grown without selection, whereas the F_2 and F_3 plants carrying F_3 and F_4 seeds undergo intensive selection for desired agronomic and quality characteristics;
(6) Advanced generation grow-outs — F_4 plants (F_5 seeds) and later generations are bulked and tested intensively for yield, other agronomic characteristics, and quality factors. Normally at this stage, plant breeders begin replicated testing at several locations to assess broad adaptation, stability under stress, and quality characteristics.

When the genetic control of a desirable characteristic is found to be complex or polygenically controlled, it should be carried as a subpopulation with primary emphasis on concentrating the genetic factors involved. This procedure as related to the main population is illustrated in Fig. 1.

Biological Evaluation

Biological evaluation of foods is essential because chemical analysis does not always reveal either the presence of antinutrients (or toxins) or how much of a nutrient (though present) is biologically available. Both of these limitations apply to legumes.

Antinutrients are usually destroyed by common cooking procedures, but it is always possible that new cultivars may contain undesirable substances that are not completely destroyed by cooking. Animal feeding trials are the standard procedure for detecting such substances, although the findings do not necessarily relate to man. In such trials, it is necessary to feed the substance at a relatively high level in the diet for a period of several weeks.

Generally, when antinutrients are present, experimental animals will either reduce their food intake (at high levels of such toxins they may refuse to eat the diet), or will grow more slowly than expected in relation to the amount of food eaten. Short feeding periods (such as may be used in bioassay procedures) and low levels of the evaluated food in the diet may not be adequate to reveal the presence of small amounts of these toxic materials.

Legumes are a source both of protein and digestible carbohydrate, and determination of the latter requires animal feeding trials. It may be possible in a research program to correlate various chemical methods with biological digestibility and consequently to use such methods as a screening procedure, but confirmation through feeding trials will always be necessary.

If the content of biologically available amino acids in legumes were known, the value of the legume protein as a supplement to any type of diet, in any quantity, could then be calculated. However, there are no satisfactory tests for the availability of even the more important amino acids. Some microbiological procedures have been developed but are not yet fully evaluated. Similarly, chemical tests for some available amino acids are not universally applicable and have serious limitations. However, a number of biological methods of protein evaluation have been in use over the years. These are:

(1) Measurement of biological value or net protein utilization (NPU) by either nitrogen balance measurements or carcass analysis;
(2) Protein efficiency ratio (PER) calculated from growth rate of the animals related to the protein consumed — with modifications such as net protein ratio (NPR) (when a group of animals on a protein-free diet is included in the test); and
(3) Relative net protein ratio (Rel. NPR) (when the last-named test includes a standard protein for comparison).

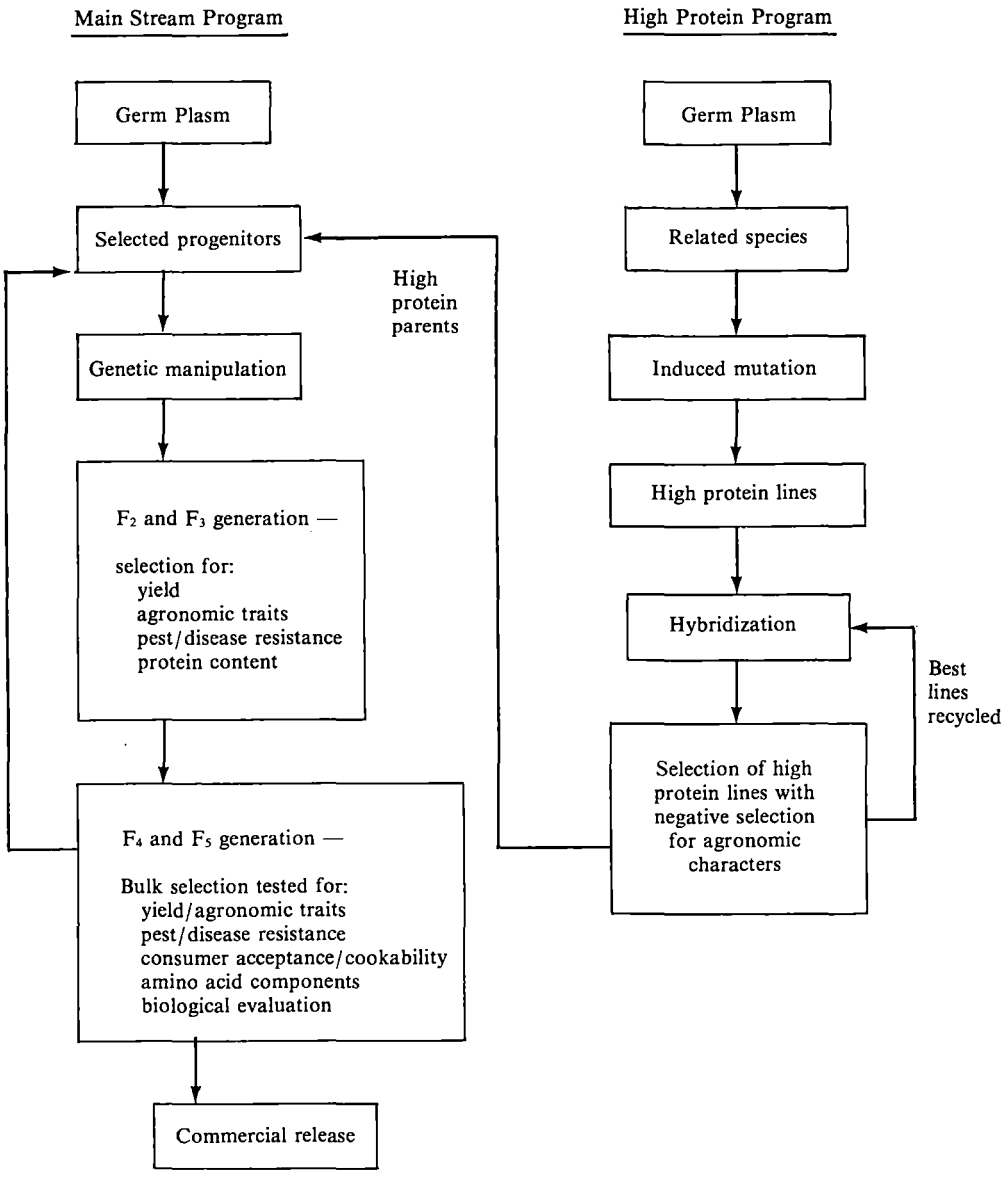


Fig. 1. Suggested structure of pulse breeding program, showing mainstream with selection for high performing lines meeting present levels of protein and protein quality, and parallel high protein program in which higher protein lines are developed for use as parents in the mainstream program. When screening methods have been adequately developed, similar support programs can be initiated for other nutrition factors.

There are also specialized methods such as gross protein value, with the chick as test animal, in which the food under examination is fed together with a basic cereal ration. Although this is a practical test simulating practical feeding conditions, it almost invariably measures the lysine content of the supplementary protein food, as cereals are limited by lysine. A number of other specialized methods of measurement have been developed, but few have come into regular use.

The drawback to all these tests, apart from the amount of material required (250 g – 1 kg of protein for duplicated assays), is that they provide information only about the amount of the limiting amino acid, without indicating which one it is. Unless the tests are repeated with various supplementary amino acids, they do not reveal which amino acid is limiting. They certainly cannot show whether all or only one of the essential amino acids is present in the amount indicated by the figure obtained, unless a multiple series of supplemented diets is examined. It is vital to bear in mind that tests carried out on single protein foods bear little relation to the value of that food when consumed as part of a diet, because of complementation between limiting amino acids.

Opinion on the relative merits of various modifications of the methods has differed over the years, and it may not be important to know values to within narrow limits of reproducibility. However, it is necessary to agree on a standardized procedure to be able to correlate results from different laboratories.

What is essential is to determine whether the amino acids present are available to man (for whom the experimental animal serves as a model), or whether they are linked in such a way as to resist the digestive enzymes. In other words, it is essential to verify any chemical estimations of amino acid content. Chemical measurements are invariably carried out after acid hydrolysis, which releases all the amino acids and so gives no information on their utilization in the body.

Though the methods recommended by the working group are directed mainly to scientists in the IARCs, it is the group's hope that they will also be of significant benefit and assistance to legume breeders and their associates in national programs, universities, and other agricultural research institutions in many countries of Africa, Asia, the Near East, and Latin America. Consequently, the methods recommended, particularly the physical and chemical methods of analysis, have been presented in considerable detail, as the journals and other publications in which the standardized methods originally appeared may not be readily available in some developing countries. It is the group's opinion that biological evaluation of legumes by laboratory animals should be confined to large central laboratories. Samples that are adequately packaged and disinfested (preferably by being subjected to below freezing temperatures for about 48 h before shipment) can safely be dispatched by mail or airfreight from a field laboratory to a central laboratory at considerably less cost than is incurred in the maintenance of an animal laboratory.

Physical and Chemical Assessment of Protein Quantity and Quality in Legumes

Sample Preparation

Whole seeds

(1) Remove extraneous matter, e.g., by the use of screens and aspiration.

(2) Grind in hammer mill or similar suitable grinder.

Note: The Cyclotec grinder (Tecatur/Udy, Inc. Boulder, Colo.) is adequate, provided larger seeds are first reduced, e.g., in a burr mill. Other suitable mills include the Christy–Norris and Falling Number Model 120 hammer mills.

Dehulled seeds

(1) Remove seed coat (testa).

Note: (*a*) Presoaking in water may be advantageous. (*b*) When yields per hectare of dehulled cotyledons are required, record the weight of dehulled material, and determine the moisture content.

(2) The material should be ground in any suitable grinder.

Storage

The digestibility of the proteins of certain legumes decreases in storage. Storage conditions that minimize deterioration should be provided where possible. Optimum storage conditions have not yet been clearly defined, although it is likely that aeration and low temperature storage will be beneficial.

The recommended procedure is to process as soon as possible after harvest, or at a constant time, the more susceptible pulses. These include the *Phaseolus* beans, and in particular those with red or black seed coats. The time elapsed between harvesting and processing should always be stated, as well as the conditions of storage.

Laboratory Analysis

Moisture determination

(1 *a*) Gravimetric — the single-stage air oven method (1 h at 130 °C ± 1 C°) is the standard procedure (3). (*b*) Drying in infrared balances, such as Ohaus or Mettler.

(2) Near infrared reflectance spectroscopy (NIRS) (4). Equipment marketed by Anacon Inc., Neotec Corporation, and Technicon Corp. is available for rapid, simple moisture estimation in ground samples.

Note: (*a*) Mean particle size (MPS) of ground material is critical — a grinder with a screen to regulate particle size is essential. MPS of ground samples within a crop should not vary by more than ±20 μm. The grinders mentioned above meet this criterion. (*b*) Thorough mixing of the ground sample is essential for accurate determination of moisture or protein by IRS analysis. (*c*) The moisture content of ground material does not necessarily reflect the moisture content of intact seeds.

Total nitrogen

(1) The recommended standard procedure is the macro-Kjeldahl test (5). A suitable sample size for materials containing 20–40% protein is 0.5 g.

(2) Micro-Kjeldahl procedures are suitable (e.g., 6,7). Block digesters that have proved to be efficient are available (8).

Note: (*a*) The ammonia realized by Kjeldahl digestion may be determined

by a number of colourimetric methods (e.g., 9). (*b*) If the sample size is less than 100 mg, caution should be exercised to ensure that the accuracy and precision of testing is not decreased as a result of increased sampling error.

(3) Colourimetric methods

(*a*) Dye-binding capacity (DBC) (10, 11) — The acid diazo dye 1-phenylazo 2-naphthol-6-sulfonic acid mono-sodium salt (commonly known as acid orange 12 or orange G) is bound quantitatively by basic amino groups in proteins. This reaction enables the dye to be used in the estimation of crude proteins in a wide range of materials.

The chief advantages of the dye-binding system are inexpensive equipment and speed of testing. The time per test is about 6 min, including grinding the sample. This compares favourably with the 60–80 min for individual Kjeldahl results. The DBC method is also applicable to batch testing — about 40 tests per hour are possible with specialized shaking equipment.

Note: (*a*) The procedure is applicable to legumes, but separate calibration against a standard Kjeldahl procedure is essential for individual crops for which calibration charts are not available. To date, DBC equipment is supplied with charts for "beans" and "peas." (*b*) Highly coloured samples have been found to give inaccurate results.

(*b*) Biuret reaction — The biuret test (12) is applicable to certain legumes. However, the use of this procedure for seeds with a highly coloured testa may lead to erroneous results due to the production of off-colours, and should be carefully evaluated by comparison with a standard Kjeldahl procedure before use.

Note: Statistical correlations between dye-binding or biuret reactions and standard Kjeldahl results should be in excess of 0.95 for protein estimations to be of an acceptable level of accuracy.

(4) Near infrared reflectance spectroscopy (NIRS) (4) — The instruments marketed by Neotec and Technicon Corporations are suitable for the screening of legumes for protein as well as moisture. The test procedure is rapid (60–75 s per test including sample grinding).

Note: (*a*) Careful calibration against the standard total nitrogen procedure is essential for each individual crop. The calibration should be renewed at 4- to 6-mo intervals for optimum accuracy. Mean particle size is important — most legumes may need to be preground, e.g., in a burr-type mill, followed by more uniform reduction in a grinder with a screen, with 1.0-mm or 0.5-mm apertures. Lentils are an exception and can be ground directly in a screen-type grinder. To date, the Cyclotec grinder (see under heading, Whole seeds, item 2) is the most suitable screen-type grinder for NIRS testing. (*b*) The initial cost per instrument is $15 000 – $20 000. This high cost is offset by high throughput, and the absence of costs for chemicals, floor space, exhaust systems, etc. where large numbers of samples (i.e., in excess of 20 000 per annum) are to be processed. (*c*) NIRS instruments do not require the employment of highly skilled technicians.

Amino acids

(1) Sulfur-containing amino acids

(*a*) Methods of hydrolysis — Efficient hydrolysis of proteins is absolutely necessary for accurate amino acid analysis. Some amino acids may be destroyed or modified during hydrolysis, and others may not be completely released. The recommended procedure involves hydrolysis with 6 *N* hydrochloric acid for 24 h at 110 °C, with suitable precautions to maximize recovery and minimize degradation (13, 14).

(*b*) The most accurate procedure for the determination of methionine and cystine is that of Spackman et al. (15). This involves the preliminary oxidation of the sample with performic acid before acid hydrolysis, and automated ion-exchange chromatography (IEC) (16, 17).

(*c*) Microbiological assay — For the approximate screening of large populations to detect abnormal levels of available methionine (but not cystine), the procedure of Kelly and Bliss (18) may be used.

Note: Microbiological assays require specialized techniques and should be entrusted to technicians experienced in this field.

(*d*) Gas–liquid chromatography (GLC) after treatment with cyanogen bromide (19–21). This new technique appears promising for the estimation of methionine. The amino acid is assayed as methyl thiocyanate by GLC. This method is significantly more rapid than IEC, but requires further evaluation for use with different types of grain legumes, as some bean varieties contain 8-glutamyl-*S*-methylcysteine, which on treatment with cyanogen bromide also yields methyl thiocyanate.

(*e*) Total sulfur (22) — The total sulfur content of legumes is simpler to determine by comparison with the IEC determination of sulfur-containing amino acids. Research is necessary to determine the efficiency of the procedure for the estimation of sulfur-containing amino acids in different types of grain legumes.

(2) Lysine — Ion-exchange chromatography (IEC)

(*a*) Lysine is determined by this technique, using a short column after acid hydrolysis. Lysine is eluted rapidly (15–25 min), and the procedure is applicable to fairly large populations (23).

(*b*) Dye-binding capacity (DBC) methods — The ordinary DBC procedure referred to above (see under heading, Total nitrogen, item 3) can be employed in relation to total nitrogen to indicate the presence of abnormally high (or low) amounts of total basic amino acids present. This procedure has been used as a screening method for high lysine in cereals. In a recent innovation to the DBC technique (24) for the determination of lysine, the DBC of the material is measured before and after treatment with propionic anhydride. This reagent reacts specifically with lysine, as distinct from total basic amino acids.

(3) Tryptophan (Pigeon pea, *Cajanus cajan* only)

(*a*) Ion-exchange chromatography — Alkaline hydrolysis is necessary for the determination of this amino acid. It is then determined on a special short IEC column (25).

(*b*) Colourimetric procedure — After alkaline hydrolysis a blue colour is developed by reaction with dimethylaminobenzaldehyde (26, 27).

Adverse factors

(1) Flatulence effect — There is evidence that the sugars raffinose and stachyose are responsible for a large part of the flatulence effect. Thin-layer chromatography (28) or GLC (29) may be used to analyze for these sugars.

Determination of lignified protein (33) (see Appendix)

Expression of Analytical Values

Moisture — express as a percentage.

Protein — express as percentage protein on a dry basis, stating the N to P (nitrogen to protein) factor used. Various factors have been used for the conversion of nitrogen values to protein. The traditional term "crude protein," used in a proximate analysis system, refers to total nitrogen × 6.25. Recent

analyses indicate that for cereals and grain legumes a more realistic measure of protein content would be given by a factor of 5.7 in place of 6.25 (31, 32).

It is essential that the conversion factor used should be clearly stated in every instance where protein content is reported.

In the final evaluation of advanced lines, it is recommended that protein values be computed in terms of protein production per hectare per day, or per crop. When abnormally high protein lines are discovered, it is necessary to investigate the extent to which the "protein" in the line is true protein, and to determine that observed increases in "protein" are not due to increases in nonprotein nitrogen.

Amino acids

(1) It is recommended that concentrations of amino acids should be expressed as milligrams of amino acid per gram of nitrogen. If values are to be expressed in relation to protein, then the nitrogen to protein conversion factor should be stated.

Collaborative Studies

Continuing collaboration is necessary among laboratories to review analytical procedures critically to ensure that methods and equipment used by each laboratory are adequately standardized and provide concordant results. The international agricultural research centres should be encouraged to collaborate with each other, and with appropriate international scientific unions, technical societies, and university and industrial laboratories, to improve commonly used chemical, physical, and biological methods and to develop new techniques with superior speed, accuracy, and reliability.

Checking the accuracy of individual laboratories

(1) A central laboratory carefully prepares samples and analyzes them for a primary constituent important in the laboratories' quality screening programs. Samples of this type are distributed from time to time to a number of collaborating laboratories, which are instructed to carry out a prescribed number of analyses for the selected constituent, by the technique usually employed. Such studies will identify any laboratory whose results may differ significantly from the mean. The reasons for discrepancies can then be sought and corrected.

Standard procedure check

(1) Several check samples are prepared and sent to collaborating laboratories with instructions to follow a set procedure. It is desirable to supply uniform reagents — especially dyes — because they can vary among different sources, and sometimes among batches from a single source.

(2) Collaborative studies, as described, assist greatly in the monitoring of reproducibility among the group of laboratories, and are also used in gaining information and experience on new techniques.

Comments

It is recognized that local plant-breeding institutions will vary widely in the magnitude both of their program and of their laboratory facilities. However, the above procedures should serve as guidelines for a systematic approach to the screening of legumes for nutritional quality improvement. The less sophisticated stations should be capable of screening early generation material for the more obvious physical attributes, and for total protein. Modern NIRS

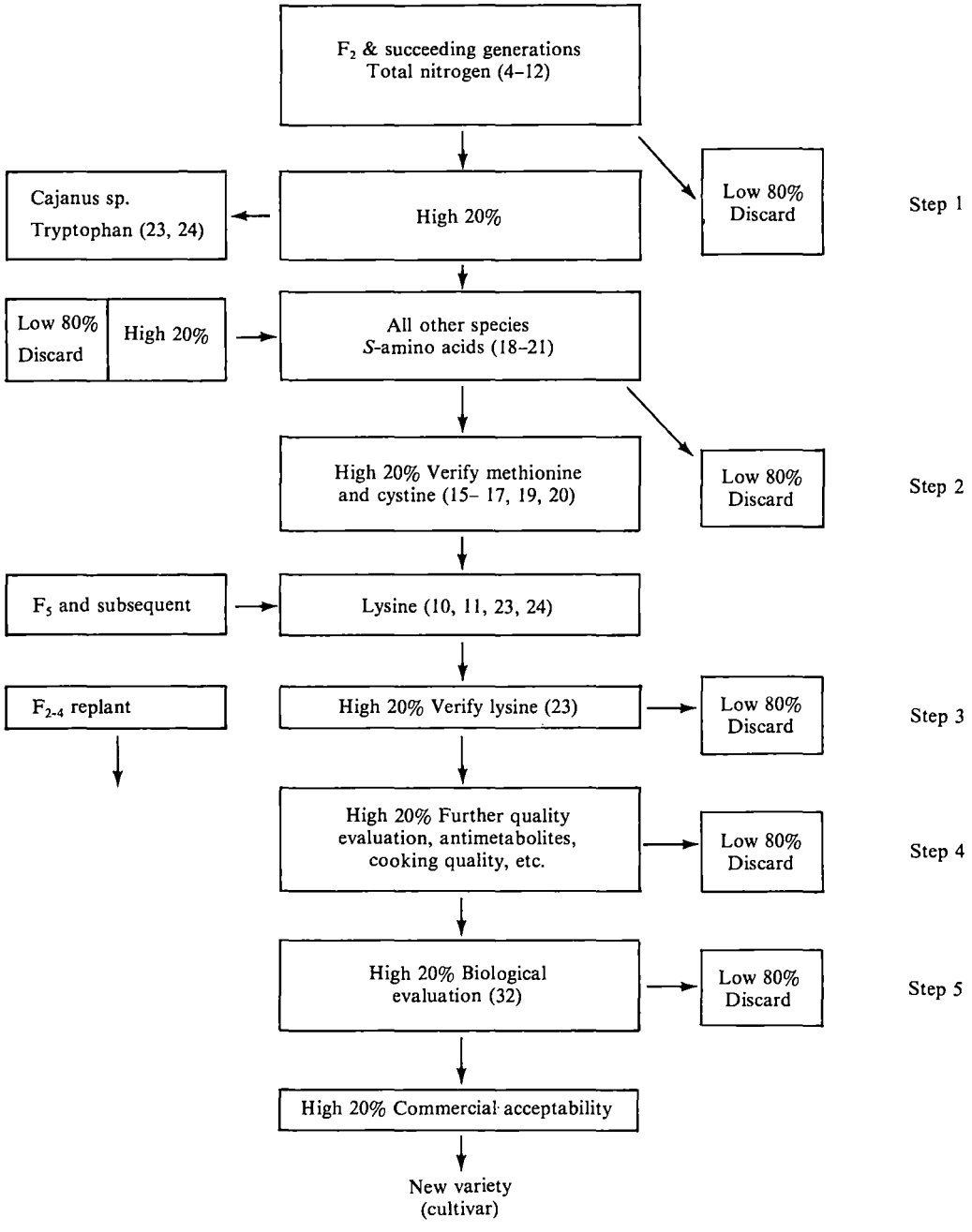


Fig. 2. Diagrammatic guide to screening procedure for legume breeding programs. The percentage eliminated at each step may vary depending on the original number of samples. A 20:80 ratio reduced 10 000 original lines to three advanced lines in five steps. A 25:75 ratio reduced 5000 to 5 in five steps and 20:80 ratio reduced 5000 to 2 in five steps (references in parentheses).

Table 3. Suitability of test procedures for use in field or central laboratories.

Test	Reference	Laboratory
Moisture	3, 4	Both
Total N	4, 5	Both
Total N	6–9	Central
Total N, DBC	10, 11	Both
S-Amino acids	13–20	Central
Lysine, IEC	23	Central
Lysine, DBC	10, 11	Both
Lysine, DBC	24	Central
Tryptophan	25–27	Central
All other tests		Central

techniques require little space, and relatively little expertise to provide facilities for screening several hundreds of lines per day for protein. The more advanced material can then be sent to larger, better-equipped stations for the performance of the more sophisticated testing. Table 2 (p. 12) summarizes approximately the numbers of lines and volume per line to be expected at certain genetic stages. Fig. 2 illustrates graphically the broad outlines of genetic and quality evaluation to be undergone in a typical program aimed at the production of high-yielding legumes with improved nutritional qualities. Certain test procedures are adaptable for use in field laboratories, whereas others are recommended for use only in more fully equipped central laboratories. Table 3 summarizes these recommendations.

Note: Various field stations may use locally developed tests for specific constituents. Future revisions of the recommended procedures will include such tests as they appear in scientific publications. It is likely, for example, that in the near future amino acids may be determined by modified NIRS techniques.

Appendix

Description of Procedures and Equipment Commonly Used in the Chemical Testing of Genetic Material

Sample Preparation

Cleaning

The bulk of the chaff, seed pods, and other seed protective material will have been removed during harvest. The remaining weed seeds and other foreign material should be removed before processing, because certain weed seeds, if present in excessive quantities and as a result of their specific composition, may introduce spurious data to the screening program. Most weed seeds can be separated from the test material by passing through sieves of various sizes. Fibrous material, such as loose seed coats, is removable by aspiration. Equipment such as the Comtar Dockage Tester and the Saginaw "Clipper" combine both sieves and aspiration, and afford a rapid (1–2 min) method for cleaning samples of grain of about 5 g or more. Large (500 g plus) samples may require subdivision before processing in the laboratory to provide an accurately blended sample. The Boerner sample divider is recommended for this purpose.

Grinding and pulverizing

Legumes differ widely in the size, shape, and texture (hardness) of their seeds. To provide a sample with as uniform a particle size as possible, all seeds should be ground in a mill that incorporates a screen through which all material passes during the grinding process. The mean particle size and particle size distribution is affected by moisture content. To minimize variations in the uniformity of ground samples, all seeds should be ground in a hammer or impeller-type mill operating at a minimum of 8500 rpm, and fitted with a 1.0-mm screen. The Tecatur/Udy Cyclotec grinder and the Christy/Norris hammer mill are both recommended. In addition, the Kamus-Slago Model 120 hammer mill is suitable.

Certain of the larger seeds may require preliminary grinding before reduction on the Cyclotec grinder. Any burr-type mill is suitable for this step. The Buhler Laboratory mill, and the Falling Number type KT-30 are the most suitable burr mills available.

Moisture — Single-stage Air Oven Method

Equipment

An oven capable of maintaining 130°C±1C°; analytical balance with precision of ± 0.001 g; moisture dishes, aluminum, 50 mm in diameter; desiccator.

Procedure

Weigh accurately 1–2 g of ground material into a previously weighed moisture dish. Place in oven at 130 °C for 65 min. Cool in desiccator and reweigh. Calculate percentage of moisture or dry matter as follows:

$$\% \ H_2O = \frac{(W_2 - W_3) \times 100}{(W_2 - W_1)}.$$

$$\% \text{ dry matter} = 100 - \% \ H_2O, \text{ or:}$$

$$= \frac{(W_3 - W_1)}{(W_2 - W_1)} \times 100.$$

where W_1 = weight of sample can empty; W_2 = weight of sample can plus sample; W_3 = weight of sample can plus sample, after drying; i.e., $W_2 - W_1$ = weight of sample.

Total Protein (Macro-Kjeldahl)

Equipment

Macrodigestion and distillation equipment, similar to LabConco or Precision Scientific, equipped with gas heaters or 650 W electric heaters; 800-ml Pyrex Kjeldahl flasks; 500-ml Erlenmeyer flasks; aspirators for bulk liquid storage; dispensers for 20, 25, and 50 ml; 20-ml pipettes; 25-ml burettes.

Reagents

(1) Concentrated sulfuric acid, H_2SO_4, 96% or 66 °C Baumé.

(2) Catalyst mix containing potassium sulfate, mercuric oxide (or titanium dioxide), cupric sulfate, and pumice (100 mesh) in the ratio 10:0.3:0.3:0.1.

(3) Sodium hydroxide, NaOH (40%) — This must include 10% sodium thiosulfate if mercuric catalyst is used.

(4) Boric acid solution, 5%, containing 4 ml of 1% bromocresol green indicator per litre.

(5) Methyl red solution, 2 ml of 1% methyl red solution per litre.

(6) Standard H_2SO_4 solution, 0.1142 *N*. Measure 15.2–15.5 ml concentrated H_2SO_4 and dilute to 5 litres.

(7) Dilute NaOH solution, approximately 0.114 *N*. Dissolve 9.2 g NaOH pellets and dilute to 2 litres.

Procedure

(1) Standardization of 0.1142 *N* H_2SO_4 — Dry 3–4 g of pure potassium hydrogen phthalate for 2 h at 130 °C. Cool in a desiccator. Accurately weigh at least four 0.5-g portions into clean 500-ml Erlenmeyer flasks. Dissolve in about 100 ml distilled (demineralized) water. Add two drops of 1% phenolphthalein indicator. Rinse a clean burette with dilute NaOH solution and titrate the phthalate samples. Calculate the normality of the NaOH using the formula:

$$N_{NaOH} = \frac{1000 \times \text{weight of phthalate (g)}}{\text{vol. of titration (ml)} \times 204.23 \text{ (equiv. of phthalate)}}$$

Replicate results must agree to within 0.0001 *N*.

Pipette 20-ml portions of dilute H_2SO_4 into Erlenmeyer flasks, add 100 ml of the methyl red indicator solution, and titrate with the standardized NaOH. Calculate the normality of the H_2SO_4 using the formula:

$$N_{H_2SO_4} = \frac{\text{Vol. of titration (ml)} \times \text{normality of NaOH}}{20}.$$

Duplicate titrations should agree within 0.0001 *N*.

Adjust the strength of the standard H_2SO_4 to exactly 0.1142 *N* by the addition of water or concentrated H_2SO_4 as necessary.

Note: (*a*) For 5 litres, 0.1 ml of concentrated H_2SO_4 increases normality by 0.0007504 *N*. (*b*) For 5 litres, 5 ml of concentrated H_2O reduces normality by 0.00011 *N*.

(2) Analysis — Accurately weigh 0.5–1.0 g of sample into the Kjeldahl flask. Add 10 g catalyst and 20 ml concentrated H_2SO_4. Digest until clear, followed by a further 20 min. Cool, dilute with 300 ml water. Add 50 ml of 40% NaOH, connect to distillation apparatus, and distil into 25 ml of boric acid solution plus 100 ml methyl red solution. This combination provides the mixed indicator. Collect 250 ml of distillate, and titrate against 0.1142 *N* H_2SO_4 until the green colour just turns purple. Record the results: 1 ml of titration = 1% protein (N × 6.25). Multiply the result by 2 if 0.5 g of sample is used. Reagent blanks should be determined daily, and the result subtracted from the sample titration. For the reagent blank, analytical grade dextrose is substituted for the sample.

Note: For anticipated protein contents of up to 15%, use 1.0 g of sample; above 15%, use 0.5 g.

Total Protein: Micro-Kjeldahl

Equipment

(1) Microdigester — This should be fitted with naked electric heating elements — Silex burners are suitable. Distillation equipment such as Parnos/Wagner, or Markham stills; 10-ml pipettes and burettes; 10- and 20-ml dispensers; 50- or 100-ml Pyrex Kjeldahl flasks; 250-ml Erlenmeyer flasks; plastic aspirators for liquid storage.

Reagents

As for macro-Kjeldahl test, but use 0.02285 *N* standard H_2S0_4 for titration. This is conveniently prepared by diluting 0.1142 N acid × 5.

Procedure

(1) Standardization — as for macro-Kjeldahl procedure.

(2) Analysis — Weigh 0.1–0.25 g of sample into Kjeldahl flasks. It is convenient to dissolve catalyst in the concentrated H_2SO_4 in the same proportion as for macro-Kjeldahl work. Use 10 ml H_2SO_4 for digestion, and glass beads in place of pumice. Digest 30 min. The use of 10 ml H_2O_2 will reduce digestion time.

Cool the digest, transfer to distillation equipment; use 10 ml boric acid solution plus 20 ml methyl red solution; add 20 ml 40% NaOH, and distil about 100 ml; titrate to purple end point; calculate the result, allowing for weight of sample.

Note: The ammonia liberated after digestion can be determined colourimetrically provided that the equipment includes a spectrophotometer, or a Technicon AutoAnalyzer. References are cited in the literature summary.

Total Protein: Dye-binding Procedure (DBC)

Equipment

Tecatur/Udy DBC equipment — Alternatively, spectrophotometer similar to Bausch and Lomb Spectronic 20; shaker; dispenser for 40 ml; filtering apparatus using fibreglass filter pads; analytical balance with precision of ±0.001g; plastic containers for dye solutions.

Procedure

Weigh 0.3–1.0 g of sample into reaction vessel. Add 40 ml dye solution, shake 3 min (high speed) or 40 min (low speed). Filter into colorimeter cell (after allowing reaction vessel(s) to stand upright for about 5 min). Record protein using table.

Note: (*a*) Adjust weight of sample according to anticipated protein content of material. (*b*) Calibrate test against Kjeldahl test for individual crops. Select 30–40 samples covering the expected range of protein. Analyze by Kjeldahl and DBC methods. Compute regression equation $Y = a + bx$, where Y = % protein DBC, and x = % protein Kjeldahl. Construct a table for future DBC analysis. A recommended text for reference to statistical computation is found in (34). (*c*) If standard visual colourimetry is used in conjunction with a spectrophotometer other than the Tecatur/Udy equipment, the sample cell is likely to be of too great a width for the employment of standard Udy dye solution. Dilute the dye by 10–100, depending on the width of the cell used.

Total Protein: Near Infrared Reflectance Spectroscopy (NIRS)

Equipment

Neotec GQA-31EL, or Technicon InfraAnalyzer NIRS reflectance spectrophotometer; Grinding mill, such as Tecatur/Udy Cyclotec.

Procedure

(1) Calibration — Select 40 samples evenly distributed throughout a range of 5–10% protein. Grind 20–25 g of sample, mix thoroughly, load sample cell, set the instrument to "*C*-cal" (GQA only) and record reflectance values of all samples ("*C*" values in the case of the GQA-31, "LOG" values for the InfraAnalyzer). Perform standard protein tests by Kjeldahl or any suitable method. Compute the calibration constants ("*K*" values) by multiple linear regression — a computer or minicomputer is necessary: the manufacturers provide computing service if required. Set the *K* constants into the instrument.

Analyze 10–12 further samples on the new calibration. Compare the mean NIRS and Kjeldahl results, and adjust the intercept as necessary.

Note: Protein calibrations should be carried out on an "as is" basis with respect to moisture content. If all samples to be analyzed are brought to approximately uniform moisture content before analysis, the instruments can be calibrated to read protein on a constant-moisture basis, e.g. dry basis.

(2) Analysis — Grind samples as for calibration, mix thoroughly, load sample cell, and record protein directly from instrument set to "%" mode (GQA only).

Total Amino Acid Analysis

Equipment

Beckman Spinco Model 120 amino acid analyzer, Technicon Auto amino acid analyzer or similar equipment, and mercury-diffusion high-vacuum pump.

Hydrolysis

Transfer 20–40 mg accurately weighed sample to 18-mm Pyrex test tube. Add 40 ml 6 *N* HCl, freeze to about −80 °C (liquid nitrogen) and evacuate to 50 μm. Allow contents to melt, seal tube. Hydrolyze at 110 °C for 24 and 72 h (two tubes). Remove HCl under vacuum (0.1 mm) in a desiccator containing NaOH. This takes about 12 h. Add 25 ml of 0.2 *N* Na citrate buffer, pH 2.2, containing Brij-35 detergent, to the residue. Filter from insoluble material, using Whatman No. 52 paper (or equivalent) using vacuum. Use portions of filtrate for amino acid determination. (Condensed from Ref. 14.)

Amino acid determination

Consult manufacturer's operation manual for specific equipment and procedures.

Lysine by Ion-exchange Chromatography (IEC)

Hydrolysis

As for total AAs — use 24-h period only.

Analysis

As for total AAs — the chromatographic column can be shortened, and the colourimetry modified to record only lysine. This allows for the determination of lysine in six to seven hydrolyzates per hour.

Lysine by DBC

Equipment

As for total protein.

Procedure

Determine total protein by DBC method. Weigh samples on a constant protein (DBC) basis — usually 60 mg protein per sample. Redetermine the DBC value. The highest values are the highest in basic amino acids, which are highly correlated to lysine itself. Standardize the procedure by including samples of known lysine content. For screening purposes, a "cut-off" level is arbitrarily set at, e.g., the high 25% range.

Lignified Protein (*Phaseolus* Beans Only)

Reagents

Acid detergent: 20 g of acetyl trimethylammonium bromide / litre of

1 *N* H_2SO_4; decahydronaphthalene, reagent grade; and acetone.

Procedure

Weigh 1 g of seed coats or 3 g cotyledons, add 100 ml of acid detergent, mix. Add 2 ml decahydronaphthalene, reflux 1 h, filter, wash with warm distilled H_2O on the filter (Whatman No. 1 paper or equivalent). Wash residue with acetone, dry at 60 °C, and determine total nitrogen by Kjeldahl. Lignified protein % = total N% × 6.25.

Trouble Shooting

This section summarizes the *principal* sources of error in the tests cited in the appendix.

Symptom	Cause
Sample preparation	
Loss of sample	Holes in screens, excessive air flow in aspiration, incorrect screens
Failure to remove detritus	Holes in screens, insufficient air flow, incorrect screens
Nonuniform particle size	Holes in grinder screen; grinder affected by sample moisture level or oil level; hammer mills with screens should not be used to grind material above 20% H_2O or above 10% oil
Moisture	
Low results	Insufficient time in oven, temperature too low, spillage of sample, analytical balance defective
High results	Temperature too high (causes charring), balance defective
High or low results	Error in calculation
Total protein, Kjeldahl	
High results	Weighing error, moisture loss in grinding, impurities in reagents
Low results	Weighing error, overdigestion (common), underdigestion, dirty burettes
High and low results	Poor sampling, poor sample preparation (particularly mixing), inaccuracy in acid standardization, carelessness in recording and calculation
Total nitrogen, micro-Kjeldahl	
As for macro-Kjeldahl	Microdigestions are more sensitive, and underheating is commonly encountered; selenium catalysts are the most difficult to control
Total protein, DBC	
High and low results	Inaccurate weighing, inaccurate dispensing of reagent, poor sampling and sample preparation (particularly mixing); variation in batches of dye, insufficient warm-up time of colorimeter; inaccurate Kjeldahl results used for standardization, errors in calculation, general carelessness
Total protein, NIRS	
High and low results	Poor sampling, poor sample preparation, in particular poor grinding and mixing, inaccuracy in Kjeldahl results used in standardization, instrument error, general carelessness

In general, most errors in AA analysis by IEC are caused by faulty hydrolysis; particular attention must be paid to this step

References

1. Dovlo, F.E., Williams, C.E., and Zoaka, L. 1976. Cowpeas. Home preparation and use in West Africa. In Graham, M., ed., Ottawa, International Development Research Centre, IDRC-055e.
2. Centro Internacional de Agricultura Tropical. 1973. Potential of field beans and other food legumes in Latin America, seminar series 2e, 25–26.
3. American Association of Cereal Chemists. 1962. American Association of Cereal Chemists. Approved methods, 7th ed. Minnesota, U.S., AACC, Minneapolis, Sect. 44-15.
4. Williams, P.C. 1975. Application of near infrared reflectance spectroscopy to the analysis of cereals and oilseeds. Cereal Chem. (U.S.), 55, 561–576.
5. American Association of Cereal Chemists. 1962. American Association of Cereal Chemists. Approved methods, 7th ed. Minnesota, U.S., AACC, Minneapolis, Sect. 46-10, 12.
6. American Association of Cereal Chemists. 1962. American Association of Cereal Chemists. Approved methods, 7th ed. Minnesota, U.S., AACC Minneapolis, Sect. 46-13.
7. Association of Official Analytical Chemists. 1970. Official methods of analysis of the Association of Official Analytical Chemists, 11th ed. District of Columbia, U.S., AOAC, P.O. Box 540, Benjamin Franklin Station, Washington.
8. Wall, L.L., Sr et al. 1975. Total protein nitrogen — evaluation and comparison of 4 different methods. J. Assoc. Off. Agric. Chem. (U.S.), 58, 811–817.
9. Crooke, W.M., and Simpson, W.E. 1971. Determination of ammonium in Kjeldahl digests of crops by an automated procedure. J. Sci. Food Agric. (England), 22, 9–10.
10. Mossberg, R. 1969. Evaluation of protein quantity and quality by dye-binding capacity: a tool in plant breeding. New approaches to breeding for improved protein. Panel proceedings series 212. In Vienna, Austria, International Atomic Energy Agency.
11. Munck, L. 1972. Improvement of nutritional value in cereals. Hereditas (Sweden), 72, 1–128.
12. Jennings, A.C. 1961. Determination of the nitrogen content of cereal grain by colorimetric methods. Cereal Chem. (U.S.), 38, 467–479.
13. Blackburn, S. 1968. Amino acid determination. New York, USA, Marcel Dekker.
14. Tkachuk, R., and Irvine, G.N. 1969. Amino acid composition of cereals and oilseed meals. Cereal Chem. (U.S.), 46, 206–218.
15. Spackman, D.H., Stein, W.H., and Moore, S. 1958. Automatic recording apparatus for use in the chromatography of amino acids. Anal. Chem. (U.S.), 31, 1190.
16. Schram, E., Moore, S., and Bigwood, E.J. 1954. Chromatographic determination of cystine as cysteic acid. Biochemistry (U.S.), 57, 33–37.
17. Moore, S. 1963. On the determination of cystine and cysteic acid. J. Biol. Chem. (U.S.), 238, 235–237.
18. Kelly, J.D., and Bliss, F.A. 1975. Quality factors affecting the nutritive value of bean seed protein. Crop Sci. (U.S.), 15, 757–760.
19. Inglis, A.S., and Edman, P. 1970. Mechanism of cyanogen bromide reaction with methionine in peptides and proteins. 1. Formation of imidate and methyl thiocyanate. Anal. Biochem. (U.S.), 37, 73–80.
20. Finlayson, A.J., and MacKenzie, S.L. 1976. A rapid method for methionine determination in plant materials. Anal. Biochem. (U.S.), 70, 297–402.
21. Ellinger, G.M., and Duncan, A. 1976. The determination of methionine in proteins by gas-liquid chromatography. Biochem. J. (England), 155, 615–621.

22. Porter, W.M., Sandhu, S.S., Axtell, J., and Kein, W.F. 1974. Evaluation of nutritive quality of legumes by an analysis for total sulfur. Crop Sci. (U.S.), 14, 652–654.
23. Mattern, P.J., Schmidt, J.W., and Johnson, V.A. 1970. Screening for high lysine content in wheat. Cereal Sci. Today (U.S.), 15, 409–411.
24. Hurrell, R.F., and Carpenter K.J. 1975. An approach to the rapid measurement of "reactive" lysine in foods by dye-binding. Proc. Nutr. Soc. (England), 35, 25A.
25. Hugli, T.E., and Moore, S. 1972. Determination of the tryptophan content of proteins by ion exchange chromatography of alkaline hydrolysates. J. Biol. Chem. (U.S.), 247, 2828–2834.
26. Rao, M.V.R., Tara, M.R., and Krishman, C.K. 1974. Colorimetric estimation of tryptophan content of pulses. J. Sci. Food Technol. 11, 213–216.
27. Spies, J.R. 1967. Determination of tryptophan in protein. Anal. Chem. (U.S.), 39, 1412–1416.
28. DeStefanis, V.A., and Ponte, J.G., Jr 1968. Separation of sugars by thin layer chromatography. J. Chromatogr. (Netherlands), 34, 116.
29. Theander, O., and Aman P. 1976. Low-molecular carbohydrate in rapeseed and turnip rapeseed meals. Swed. J. Agric. Res. (Sweden), 6, 81–85.
30. Tkachuk, R. 1969. Nitrogen to protein conversion factors for cereals and oilseed meals. Cereal Chem. (U.S.), 46, 419–423.
31. Holt, N.W. 1976. Amino acid and non-protein nitrogen content of grain legumes. Ph.D. thesis, Department of Crop Science, University of Saskatchewan, Saskatoon, Canada.
32. Protein Advisory Group Bulletin. 1975. New York, U.S., United Nations Systems (N.Y.) V(2), 40–43.
33. Goering, H.K., and Van Soest, P.J. 1970. Forage fibre analyses. In Agricultural Handbook No. 379, 387–398.
34. Steel, R.G.D., and Torrie, J.H. 1960. Principles and procedures of statistics. New York, U.S., McGraw-Hill Book Company.

Chapter 2

Biological Evaluation of Protein Quality of Legumes

Amino Acid Score (Chemical Score)

The amino acid score is a useful guide to the potential nutritive value of the protein. It is a comparison of the amount of the limiting amino acid (the combination of methionine plus cystine for legumes) in the protein, determined by chemical methods, with the level of that amino acid in the 1973 joint FAO/WHO provisional reference pattern (1). When the complete amino acid pattern is known, the amino acid score is calculated as: content of limiting amino acid in test protein/content of same amino acid in reference protein.

The lowest score indicates the limiting amino acid, and its numerical value correlates directly with values of protein quality obtained with young growing rats (2, 3). The limiting amino acid in cereals is lysine, and in legumes is the combination of methionine and cystine. When equipment for complete amino acid analyses is lacking, measurement of the appropriate amino acid, and comparison with the provisional reference pattern provides the amino acid score (3). In the amino acid score method, the assumption is made that amino acid is fully available nutritionally. This assumption is not always true for various reasons, and actual protein values are sometimes lower than predicted by the amino acid score.

Rat Bioassays

The relative net protein ratio (relative NPR) (4, 5) is recommended as the first screening assay for protein quality when quantities of the test legume are limited. At a later stage, when adequate quantities of the test legume are available, a second assay — the relative protein value (RPV) — may be performed (4, 6, 7). Both assays are designated "relative" because they are related to a reference standard protein.

Preparation of samples

(1) Weigh appropriate amount of dry pulse (see p. 16, Storage); add five parts water to one part pulse in suitable cooking equipment and soak for 12–15 h. Pour off the soaking water. (Any departure from the recommended procedure should be reported.)

(2) Add sufficient water to give a layer 1 in. (2.54 cm) deep on top of the soaked pulse.

(3) Cook in an autoclave at 15 psi as indicated below. Time is measured from the moment the pressure reaches 15 psi.

	Time (min at 15 psi)
Cowpeas	10
Phaseolus	20
Pigeon peas	20
Chick-peas	20
Broad beans	20
Lentils	10
Dry peas	10–15

(4) Chop the cooked sample in a food blender to reduce particle size. Spread the blended pulse in a suitable drying apparatus and dry at a temperature not exceeding 55 °C.

(5) Grind dried pulses in a suitable mill (take samples for nitrogen analyses and trypsin inhibitor activity).

(6) Carry out nitrogen analysis by Kjeldahl procedure.

Diets

The basal diet has the following composition:

	g/100 g diet
Maize starch	86
Vegetable oil	10
Salt mixture (8)[a]	3
Vitamin mixture (6)	1

[a]The salt mixture should be supplemented with zinc to 50 ppm.

Relative NPR

In preparation of diets, protein is added to give a level of approximately 8% (1.3% nitrogen) at the expense of maize starch. Casein is the reference protein (ANRC-high nitrogen casein from Sheffield Chemical Co., Norwich, N.Y. 13815, USA). If the test sample has a high fat or fiber content, consideration should be given to equalizing the level of these substances in the standard and test diet.

Three diet groups are included in the relative NPR assay: a standard or reference protein (casein) group; a test protein group; and a protein-free basal diet group.

Animals

Male weanling rats of a single strain, 21–23 days old, are fed the reference casein diet for 2 days. Rats are then distributed in groups of eight so that the mean weights of the groups are within 1 g of each other. The weight variation of rats within a group should preferably not exceed 10 g.

Rats are placed in individual screen-bottom cages preferably in an air-conditioned room maintained at 24–25 °C. Food and water are provided ad libitum. Food cups designed to reduce spoilage should be used. Paper or cardboard is placed under cages to catch spilled food, which should be recovered daily. Food consumption and weight gain of rats are determined weekly for 14 days.

Table 4. Data on initial weight, weight changes, and protein consumed, for individual rats in a 14-day NPR assay.

Rat no.	Initial wt (g)	Final wt (g)	Wt change (g)	Food consumed (g)	Protein consumed (g)
Casein diet, 8% protein					
1	48	81	+33	119	9.52
2	42	60	+18	83	6.64
3	39	70	+31	107	8.56
4	45	75	+30	110	8.80
5	41	75	+34	112	8.96
6	35	62	+27	100	8.00
7	36	60	+24	97	7.98
8	38	64	+26	101	8.08
Soy protein diet, 8.4% protein					
9	48	59	+11	81	6.80
10	42	46	+ 4	54	4.54
11	39	45	+ 6	67	5.63
12	45	52	+ 7	70	5.88
13	40	44	+ 4	57	4.79
14	35	42	+ 7	51	4.28
15	37	45	+ 8	63	5.29
16	38	43	+ 5	55	4.62
Nonprotein diet					
17	47	36	-11		
18	42	33	- 9		
19	39	30	- 9		
20	45	31	-14		
21	40	29	-11		
22	36	28	- 8		
23	37	27	-10		
24	38	27	-11		

Calculation of NPR

NPR may be calculated in two ways. In the simpler method, the mean weight gain for the test animals (WGT) plus the mean weight loss for the group fed a nonprotein diet (WLB) is divided by the mean protein consumption for the test group (PT).

In the second method, NPR values are obtained for each animal fed the test diet. In the assignment of animals to different diets, rats are selected for equal initial body weights. An example is given in Table 4. The first rat in each of the three groups weighed 47–48 g; the second rat in each group weighed 42 g and so on. Calculation of NPR values is given in Table 5. The NPR for rat no. 1 is calculated as follows: WGT (33 g) plus the WLB of the corresponding rat fed the nonprotein diet (11 g) is divided by the protein consumed by rat no. 1 (9.52 g) giving an NPR of 4.62. Individual NPR values give a measure of the error of the assay. The relative NPR for soy protein is: $\frac{3.23}{4.57} = 0.71$.

$$\text{Calculation of relative NPR} = \frac{\frac{\text{WGT} + \text{WLB}}{\text{PT}}}{\frac{\text{WGR} + \text{WLB}}{\text{R}}}$$

where WGT = weight gain of test protein group; WLB = weight loss of basal (protein-free) diet group; WGR = weight gain of reference protein group; T = nitrogen consumed by test protein group; R = nitrogen consumed by reference protein group.

Table 5. Calculation of NPR for casein and soy protein.

Rat no.	Casein	Rat no.	Soy protein
1	$\frac{33+11}{9.52} = 4.62$	9	$\frac{11+11}{6.80} = 3.24$
2	$\frac{18+9}{6.64} = 4.07$	10	$\frac{4+9}{4.54} = 2.86$
3	$\frac{31+9}{8.56} = 4.67$	11	$\frac{6+9}{5.63} = 2.66$
4	$\frac{30+14}{8.80} = 5.00$	12	$\frac{7+14}{5.88} = 3.57$
5	$\frac{34+11}{8.96} = 5.02$	13	$\frac{4+11}{4.78} = 3.14$
6	$\frac{27+8}{8.00} = 4.38$	14	$\frac{7+8}{4.28} = 3.50$
7	$\frac{24+10}{7.98} = 4.26$	15	$\frac{8+10}{5.29} = 3.40$
8	$\frac{26+11}{8.08} = 4.58$	16	$\frac{5+11}{4.62} = 3.46$
Mean NPR = 4.57±0.12		Mean NPR = 3.23±0.11	
		Relative NPR for soy protein = $\frac{3.23}{4.57} = 0.71$	

RPV Modified (or Slope Ratio Assay)

Principle

This method is slightly modified from the RPV method given in (4).

The RPV method is useful for the biological evaluation of advanced lines. In this assay the rate of body weight change of rats fed various levels of the test protein is compared with that obtained with animals fed a reference protein. Ideally the body weight response should be linear over the range of protein intakes tested. Note that the accuracy of the estimate depends on number of animals used, the variations around the regression lines, and the range of protein intakes included in the test. The wider the range of protein levels tested, the more accurate will be the result, providing the regression is linear. The appropriate levels of test protein to be used in the assay depend on the quality and quantity of protein in the test material.

Animals

Weanling rats of a single strain, 21–23 days old, are assigned to groups of four animals (two of each sex if both males and females are used), one group for each test protein level. The weight variation of the animals should not be greater than 10 g. The rats should be distributed among the various test groups so that mean weights of the groups fall within 1 g of each other at the start of the assay.

Diets

To achieve the levels of dietary protein intake described below, the diets are formulated on the basis of Kjeldahl nitrogen content of the air-dry material. The composition of the protein-free basal diet is given in the relative NPR assay. An alternative diet useful for evaluation of cereal protein quality has also been described (9).

The reference protein diet is formulated by substituting the appropriate amounts of the reference protein for starch in the protein-free basal diet. Casein is the reference protein (ANRC-high nitrogen casein). Reference protein is fed in each assay at three dietary nitrogen levels: 0.3, 0.8, and 1.3% (equivalent to 2, 5, and 8% protein on an N × 6.25 basis).

Aberrant results may be obtained if the levels of protein selected are too high or too low (9). Unusually high levels of protein intake result in nonlinear body weight responses, thus distorting the slope of the curve downward and underestimating the protein quality. When preparing diets, consideration may be given to the need to (*a*) adjust the oil content to take into account the lipid level of the test material, and (*b*) equalize the fiber content of the diets by use of Alphacel or similar materials.

The test protein diet is prepared in a manner similar to the reference protein diet. Generally, the test protein should be fed at nitrogen levels[1] of 0.64, 1.28, and 1.92%; again the test material is incorporated into the diet at the expense of maize starch.

Procedure

The rats are placed in individual cages and fed the reference protein diet at a level of 1.3% N for 2 days. The rats are then assigned four per group, equalized for weight and sex distribution, as described above. One group is fed the protein-free basal diet; three groups are fed the reference protein, each at its own prescribed nitrogen level. (Obviously for each additional test protein, three additional groups of rats would be needed.) All the rats are fed ad libitum for 14 days. Food consumption and weight gain of the rats are determined at least weekly. Paper or cardboard is placed under cages to permit collection of spilled food, which is freed of feces and weighed daily.

Calculation of test values

A table of weight changes, food intake, and calculated nitrogen intake is prepared for each animal. Regression of body weight change on nitrogen intake is calculated for the reference and test proteins. The data for the group of rats fed the basal nonprotein diet should be included in the calculation of the slopes of both the reference protein and test protein. A satisfactory approximation can usually be obtained by plotting the mean group data for weight change versus nitrogen consumed and drawing the line by inspection. Appropriate statistical methods can also be used for calculating the results, testing the linearity of the assay, and estimating the errors of the assay results.

The relative protein value is calculated as follows:

$$\text{RPV} = \frac{\text{Slope of the test material}}{\text{Slope of the reference material}}.$$

Dry Matter and Protein Digestibility

Measurements of feed intake are made during the last 7 days of the NPR assay, and all fecal matter is collected. This is dried, weighed, and analyzed for nitrogen. Dry matter digestibility is calculated by subtracting from feed intake (0% moisture) the dry weight of feces and dividing the difference by weight of feed intake.

[1]Some laboratories use slightly different levels in this test. The levels should always be stated, and also the conversion factor used, in reporting results of tests.

$$\% \text{ dry matter digestibility} = \frac{\text{Feed intake (g)} - \text{fecal wt (g)} \times 100}{\text{Feed intake (g)}}.$$

Apparent protein digestibility is calculated by the following formula:

$$\% \text{ apparent protein digestibility} = \frac{\text{N intake} - \text{fecal N} \times 100}{\text{N intake}}.$$

True protein digestibility can also be calculated by allowing for the fecal N in the group of rats fed a nonprotein diet in the NPR assay.

Test for Gross Toxicity

Twelve young rats are fed ad libitum on a diet containing at least 90% of the cooked pulse flour supplemented with 0.2 g methionine/100 g diet. The diet must contain adequate amounts of calories, vitamins, and minerals, and 5% vegetable oil.

The animals are fed for a period of 6 weeks, with water available at all times. A commonly used variety of the same pulse food should be fed in the control diet.

The average weight of the pancreas relative to average body weight of rats in test groups is then compared with similar weights of rats in the control group.

References

1. Food and Agriculture Organization of the United Nations/World Health Organization. 1973. Energy and protein requirements: reports of a joint FAO/WHO *ad hoc* expert committee. WHO technical reports series no. 522. Geneva, Switzerland, World Health Organization. *Also as* FAO nutrition reports series no. 52. Rome, Italy, Food and Agriculture Organization.
2. Block, R.J., and Mitchell, H.H. 1946. The correlation of amino acid composition of proteins with their nutritive value. Nutr. Abstr. Rev. (England), 16(2), 249–278.
3. McLaughlan, J.M., Rogers, C.G., Chapman, and Campbell, J.A. 1959. Evaluation of protein in foods. IV. A simplified chemical score. Can. J. Biochem. Physiol. (Canada), 37, 1293.
4. Protein Advisory Group. 1975. Guideline (No. 16) on protein methods for cereal breeders as related to human nutritional requirements. PAG Bulletin, vol. 5, no. 2, June 1975.
5. Bender, A.E., and Doell, B.H. 1957. Biological evaluation of proteins: a new aspect. Br. J. Nutr. (England), 11, 140.
6. Hegsted, D.M., and Chang, Y.O. 1965. Protein utilization in growing rats. I. Relative growth index as a bioassay procedure. J. Nutr. (U.S.), 85, 159–168.
7. Hegsted, D.M., and Juliano, B.O. 1974. Difficulties in assessing the nutritional quality of rice protein. J. Nutr. (U.S.), 104, 772–781.
8. Berhhart, F.W., and Tomarelli, R.M. 1966. A salt mixture supplying National Research Council estimates of the mineral requirements of the rat. J. Nutr. (U.S.), 89, 495–500.
9. Bressani, R., Elías, L.G., del Busto, J.A., and Gupman, M.A. 1974. Protein quality screening assay for cereal grains and legume foods. Presented at the ad hoc meeting working group of protein methods for cereal breeders held in Civdad Obregón, Mexico, 17–20 April 1974. Sponsored by IDRC/PAG/CIMMYT/IUNS.
10. McLaughlan, J.M., and Campbell, J.A. 1974. Methodology for evaluation of plant proteins for human use. In Hulse, J.H., and Laing, E.M., ed., Nutritive value of triticale protein. Ottawa, International Development Research Centre, IDRC-021e.

Chapter 3

Screening Methods to Determine Cooking Quality

In the methodology to select and evaluate beans, "cooking quality and culinary characteristics" refer to the minimum time required to soften the seeds, and to bean size, absence of fissures, thickness of the cooking broth, and other visually apparent qualities (1–3).

Size

The method (2, 3) involves separation of the beans by use of a set of standard wooden-frame sieves, of which the wire mesh web has openings of graded sizes. Grading is carried out on a 3-kg sample of recently harvested beans, as well as on beans after they have been soaked in tap water for 18 h at room temperature.

Hydration Coefficient

Eight 100-g samples of size-graded beans are soaked in tap water at room temperature for periods of 4, 8, 12, 16, 18, 20, 22, or 24 h at a bean–water ratio of 3:10. After soaking the specified time, the beans are immediately removed from the water, drained, and weighed (2, 3). The calculation is as follows:

$$\% \text{ hydration coefficient} = \frac{\text{Initial wt (g)} + \text{wt of imbibed water (g)}}{\text{Initial wt (g)}} \times 100.$$

Cooking Time

Heat 150 ml of distilled water to boiling in a 250-ml Erlenmeyer flask under prevailing conditions of pressure and temperature; 100 recently harvested bean seeds are added and the flask is covered with wet filter paper to reduce evaporation.

Cooking time is measured to the point when 50% of the beans are split. This time has been found to be about 15 min (2–4).

Hardness of the Seed

Seed hardness is measured in both raw and cooked seeds, following the cooking method described above. A sample of at least 20 seeds is used for the puncture test. The puncture test is carried out with a locally built texture-testing machine (2, 4, 5), equipped with an automatic digital readout that registers the electromagnetic force applied to make a punch penetrate the sample. (The machine is called a penetrometer; see following section.) The principle of the test is similar to that applied in the Instron Universal Testing Machine when adapted to the puncture test (3). A circular flat-faced steel punch of 0.126-cm diameter is attached to the inverted load cell of the instrument. Centred directly beneath is a small wooden block that has a hole of 0.30-cm diameter drilled through it. A countersink on the upper side of the block helps to centre the cooked bean and hold it in place when the punch applies pressure. The puncture force is expressed in grams according to the calibration of the apertures (2, 4, 5).

Thickness of Cooking Broth

Thickness of the cooking broth is determined in the liquid obtained from the "cooking time" test. Thickness is evaluated by the total solids content (6) or by use of a viscosimeter.

Screening Methods for Processing Quality

The following tests are performed to evaluate the suitability of different bean types for use in the manufacture of processed products. They are carried out in processed and precooked materials obtained from the different samples.

Damaged Starch

The damaged starch content of the samples is determined by the enzymatic method described by Farrand (5). The damaged starch is expressed as a fraction of the total starch, which is determined by the standard AOAC (Association of Official Analytical Chemists) (7) method applicable to legume seeds.

Amylose

The amylose content of the different materials is determined according to Williams (8).

Viscosity

The viscosity of the materials is determined with a Brabender Amylograph and following the method used by Molina et al. (10).

Water Absorption Capacity

The water absorption capacity is evaluated with a Brabender Farinograph, at a standard viscosity value, by the method described by Molina et al. (10).

Viscosity or Thickness of Bean Puree

The viscosity or thickness is measured in bean purees prepared in standard fashion from the different varieties, or combinations of them. When viscosity is measured in a product prepared from precooked-dehydrated flours, a standard, prepared from whole grains by a similar formulation is run at the same time. The viscosity or thickness of the purees is measured at room temperature with a suitable viscosimeter, such as a rotating-cylinder viscosimeter.

Emulsifying Capacity

The emulsifying capacity of the samples is evaluated by the method described by Swift et al. (9). Correlations between the values obtained and the content of different protein fractions of the individual samples (protein fractionation mentioned previously) is looked for.

Quality Control Tests

The quality control tests carried out in prepared products based on legume seeds are those described in the Pan American Health Organization – Adolfo Lutz Institute Food Sanitary Standards (11). As well, specific quality control tests for specific products such as solids-in-cooking water for pasta products, not included in the Central American Official Standards cited above, are carried out by standard methods of the AACC (American Association of Cereal Chemists) (13).

Colour

When colour is a determinant of acceptability of bean-based products, it is measured with the Lovibond Tintometer and the general method described by Molina et al. (14).

Dehulling

The dehulling operation is carried out as a day operation by abrasion of the seed, with equipment similar to the Ce-Co-Co Barley and Wheat Polishing (debranning) Machine and Carter Dockage Tester, already used for this purpose with some success (15). In *Vigna* and similar species, dehulling is carried out by crushing the seed followed by air separation of the hulls (16). Alternatives of sifting a whole legume flour to remove the peels or hulls should be examined.

Water Activity

Water activity determination is carried out in those products capable of being prepared as an intermediate moisture product (i.e., bean purees). Water activity determinations are carried out by the vapour pressure manometric technique (17).

Chemical Methods

All samples are routinely analyzed for moisture, crude fiber, and protein content following techniques described in the AOAC manual (9). Likewise, the samples are analyzed for lignified protein by the acid detergent fiber method of Goering and van Soest (17), and for total sulfur by the method of Mottershead (18). Other subjects of interest are tannins, trypsin inhibitor activity, and in vitro protein digestibility.

Protein in beans is analyzed for lysine, tryptophan, methionine, cystine, and threonine content, and occasionally protein fractionation studies are conducted. The following fractionation method is used: a sample of ground raw beans is extracted with a salt solution to yield two fractions, one being soluble protein and the other residual protein. The soluble protein, consisting of the albumin and globulin fractions, is further fractionated into albumin and acid-soluble and acid-insoluble globulin fractions.

Biological Methods

Sample Preparation

Raw whole bean samples are washed with water, then soaked for 16 h at 4 °C in three parts water to one part beans. After soaking, excess water is drained off and fresh water added for pressure cooking. The water should cover the beans to about 1 in. (2.54 cm) above the bean surface. This procedure is carried out in special containers developed for bean cooking. Cooking is carried out under 16 psi (212 °C) for specific times, depending on the legume food under study. For *Phaseolus* 20–30 min is required, for cowpeas 10–15 min, and for *Vigna* 15–20 min. After cooking, the samples are dried with the cooking liquor and ground to a flour, which is analyzed for nitrogen, trypsin inhibitor activity, and tannins.

References

1. Elías, L.G., Bressani, R., and Flores, M. 1973. Problems and potentials in storage and processing of food legumes in Latin America. In Potentials of field beans and other food legumes in Latin America. Cali, Colombia, Centro Internacional de Agricultura Tropical (CIAT), 1973. Series seminars no. 2E, 52–87.
2. Ruiloba, E. de 1973. Efecto de diferentes condiciones de almacenamiento sôbre las caracterîstiçãs físico-químicas y nutricionales del frijol (*Phaseolus vulgaris*). Tesis de Maestria, Curso de Postgrado en Ciencias y Tecnologia de Alimentos, CESNA/ Universidad de San Carlos de Guatemala, 1973.
3. Gómez Brenes, R.A., Elías, L.G., Ruiloba, E. de, and Bressani, R. 1974. Desarrollo y uso de un instrumento de laboratorio para medir la dureza del grano de frijol. Presented at the 20th Reunión del Programa Cooperativo Centro Americano para el mejoramiento de cultivos alimenticios (PCCMCA). San Pedro Sula, Honduras. 11–15 Feb 1974.
4. Burr, H.K., Kon, S., and Morris, H.J. 1968. Cooking rates of dry beans as influenced by moisture content and temperature and time of storage. Food Technol. (U.S.), 22, 336.
5. Farrand, E.A. 1964. Flour properties in relation to the modern bread processed in the United Kingdom, with special reference to alpha-amylase and starch damage. Cereal Chem. (U.S.), 41, 98–110.
6. Elías, L.G. 1974. Efecto del almacenamiento sôbre algunas caracterîstiçãs físico-químicas y culinarias del frijol. Presentado en la XX Reunión Anual del Programa Cooperativo Cèntro Americano para el Mejoramiento de Cultivos Alimenticios (PCCMCA). San Pedro Sula, Honduras, 11–15 Feb 1974.
7. Association of Official Analytical Chemists. 1970. Official methods of analyses, 11th ed. Washington, D.C., Association of Official Agricultural Chemists.
8. Williams, P.C., Kuzina, F.D., and Hlynka, I. 1970. A rapid colorimetric procedure for estimating the amylase content of starches and flours. Cereal Chem. (U.S.), 47, 411–420.
9. Swift, C.E., Lockett, C., and Fryar, A.J. 1961. Comminuted meat emulsions. The capacity of meats for emulsifying fat. Fat Technol. 15, 468–471.
10. Molina, M.R., Mayorga, I., and Bressani, R. 1976. Production of high-protein quality pasta products using a semolina–corn–soy flour mixture. II. Some physico-chemical properties of the untreated and heat-treated corn flour and of the mixtures studies. Cereal Chem. (U.S.), 53, 134.
11. Oficina Sanitaria Panamericana. 1972. Normas Sanitarias de Alimentación, vol. 3, Revisadas. Instituto Adolfo Lutz. 1264.

12. Kon, S., Brown, A.H., Ohanneson, J.E., and Booth, A.N. 1973. Split peeled beans: preparation and some properties. J. Food Sci. (U.S.), 38, 496–498.
13. Molina, M.R., Gudiel, H., de la Fuente, G., and Bressani, R. 1974. Use of *Phaseolus vulgaris* in high-protein quality pasta products. Presented at the Fourth International Congress of Food Science and Technology, Madrid, Spain, 23-27 September 1974.
14. Molina, M.R., Batten, M.A., Gómez Brenes, R.A., King, K.W., and Bressani, R. 1976. Heat-treatment. A process to control the development of the hard-to-cook phenomenon in the black beans (*Phaseolus vulgaris*). J. Food Sci. (U.S.), 41, 661.
15. Labuza, T.P., Acott, K., Tatini, S.R., Lee, R.Y., Flink, J., and McCall, W. 1976. Water activity determination: A collaborative study of different methods. J. Food Sci. (U.S.), 41, 910–917.
16. Moreyra, R.F. 1974. Influencia de variedad de maíz y granulometría de la harina sôbre las propiedades fisicoquímiçãs y nutricionales de mezclas de maíz-leguminosa-semolina, utilizadas para fabricaciôn de pastas alimenticias. M.Sc. tesis, Universidad Iberoamericana, México, D.F., México.
17. Goering, H.R., and van Soest, P.J. 1970. Forage fiber analysis. In Agricultural Handbook No. 379. Agricultural Research Service. U.S. Dep. Agric., Washington, D.C., 387–398.
18. Mottershead, B.E. 1971. Estimation of sulphur in biological materials using the technicon auto-analyzer. Lab. Pract. (England), 20, 483.
19. Harper, J.M., and Lorenz, K. 1974. Production and evaluation of salt bed roasted full-fat soy flour. Lebensm.-Wiss. Technol. (Switzerland), 7, 268–272.

Chapter 4

Mechanical Device (Penetrometer) for Measuring the Degree of Hardness in Beans

This instrument measures the resistance offered by a bean seed when penetrated by a flat steel probe connected to an electromagnet. The force exerted by the latter increases as the amperage increases through a rheostat connected to a constant-speed electric motor. When the force exerted by the electromagnet overcomes the resistance of the bean seed and the probe pierces the bean, the whole electric system is automatically disconnected and the reading can be recorded. Fig. 3 illustrates the essential parts of the apparatus.

Calibration

A triple-beam single-pan balance was used to calibrate the penetrometer. The pan was placed under the pick and readings were taken with loads of 100, 200, 300, 400, 500, 600, 700, and 800 g. A direct relation was found between the readings and the grams-force exerted, with a straight line correlation of 0.98 when uncorrected averages were used.

Use of the Penetrometer in Research

Hardening of beans during storage

For this study, black beans (*Phaseolus vulgaris*) were stored at temperatures of 4, 25, and 32 °C. Moisture content of samples at each temperature was 8, 11, and 17%. Hardness of beans stored under these nine sets of conditions was measured after 2, 4, and 6 mo storage. The sample size in all tests was 20 seeds per treatment.

The beans were soaked in water at room temperature for 18 h and then tested on the penetrometer. Hardness increased from 2.53 to 3.28 as storage

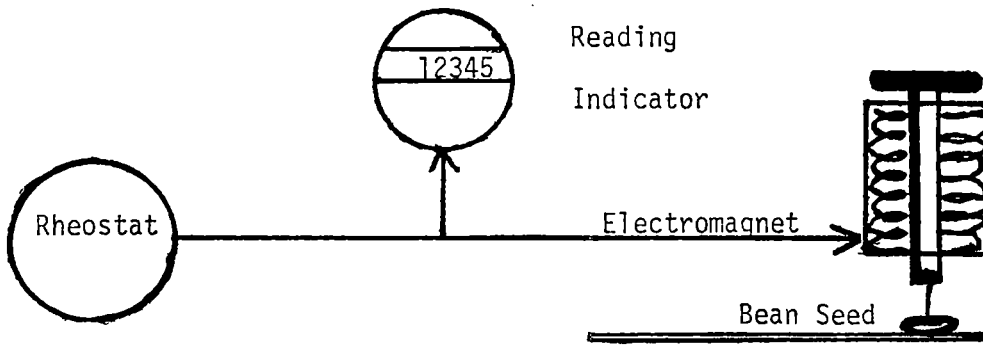


Fig. 3. A diagram illustrating the essential parts of the mechanical device (penetrometer) for measuring the degree of hardness in beans.

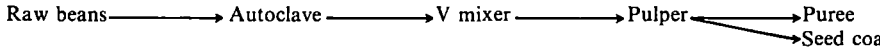
temperature and moisture content of the samples increased; also, small seeds were harder than large seeds.

The hardness of beans similarly soaked for 18 h and then cooked for 15 min in an open container also increased after storage at increased moisture content and temperature.

Bean samples soaked for 18 h were cooked in open containers and a sample was taken every 5 min to determine hardness. It was found that, with increased hardness of the seeds during storage, it became necessary to cook them from 15 to 65 min to attain the same degree of softness as that of control lots that had been stored in a freezer at low moisture content.

Preparation of bean puree

The following procedure was followed:



In this study the penetrometer was used for measuring hardness of beans after 5, 10, 15, 20, and 25 min of cooking in the autoclave. Cooking for 10–15 min gave optimum consistency for preparing a puree without loss of puree in the discarded coats. The instrument can be useful in quality control testing because adequate cooking time can be determined to obtain the desired consistency even in hard raw seeds.

Alkaline cooking of soybeans and corn

To determine the effect of alkaline cooking on the hardness of whole soybeans, samples of beans mixed with corn were cooked for 15, 30, 45, 60, 75, and 90 min with 0, 1, 2, and 3% calcium hydroxide. At the end of each treatment the whole soybeans were separated and their hardness was measured with the penetrometer. Cooking for 15 min was optimum to attain the desired softness in soybeans, regardless of the $Ca(OH)_2$ concentration.

Possible Improvement of the Penetrometer

Numerous devices are used to measure hardness, but because of the simplicity, accuracy, and low cost of the penetrometer described, it should be improved to widen its application. It would be desirable, for example, to equip the penetrometer with a more powerful electromagnet to enable it to test hardness of raw unsoaked seeds. The apparatus could be within the reach of research institutions and universities with limited resources.

Chapter 5. Background Papers

Breeding Strategy for the Nutritional Improvement of Pulses

G.C. Hawtin, K.O. Rachie, and J.M. Green

The successful development of high-yielding cereal varieties in recent years has served to emphasize the value of plant breeding in increasing world food production. Efforts are now underway, in both international and national programs, to achieve similar successes with a wider range of crops. Because of their value both nutritionally and in farming systems, the pulses are now considered to be one of the most important groups of crops requiring urgent attention. The majority of pulses grown in the developing world are unimproved local cultivars, and the potential for the genetic improvement of these species is enormous.

Nutritionally the pulses are important primarily as a source of protein, although their contribution to the carbohydrate, mineral, and vitamin fractions of the diet can often be significant. From an agricultural standpoint the pulses are valuable in the maintenance of soil fertility, primarily through their ability to fix atmospheric nitrogen. They also frequently play an important role in crop associations and rotations, in the stabilization of soils, in generating farmer incomes, and in agricultural diversification.

Breeding Objectives

It is generally accepted among breeders that the most important objective in pulse improvement is the increase and stabilization of seed yields. Often, this can be best achieved by breeding for resistance or tolerance to factors that adversely affect yield in addition to raising the potential yielding capacity of the crop. The pulses are subject to a wide range of hazards including pests, diseases, weeds, drought, waterlogging, cold, heat, salinity, and lack of available nutrients. Cultivars exhibiting resistance to these have a greater chance of achieving their maximum potential. Other agronomic characteristics may also be considered by breeders for genetic improvement, such as an increased ability to fix nitrogen in symbiotic association with suitable *Rhizobium* bacteria, a plant growth habit suitable for mechanized harvesting, nonshattering of pods, and a maturity period designed to optimize production in a given agricultural situation.

Since the primary use of the pulses is as a human food, nutritional quality is of concern to most breeders. Accepting that the improvement and stabilizing of seed yield and yield-related characteristics constitute the main objectives of most programs, there is considerable scope for nutritional improvement, especially through the international programs. Even in cases where the resources required to undertake positive nutritional improvement are not available, care should be taken that the nutritional level of released cultivars does not fall below an acceptable standard.

Nutritional Objectives

Nutritional factors can be broadly classified into two groups: (*a*) those factors, such as cooking quality and organoleptic properties that are of direct concern to the consumer and that must be at an acceptable level if a variety, however agronomically superior, is to be adopted by the farmer; (*b*) factors that may be desirable nutritionally, e.g., high protein quantity and quality, but that are generally of little concern to the farmer in the absence of a system of premium pricing. If varieties having superior food value are to be widely accepted by the farmer, it is generally necessary that any such improvements be accompanied by a higher or more stable yield or improved agronomic characteristics.

Of the wide range of possible nutritional factors in a breeding program, the choice of ones to concentrate on will depend on (*a*) their relative importance as determined by nutritionists, (*b*) the availability of suitable screening methods, and (*c*) genetic considerations.

Screening Methods

Before the positive genetic improvement of a nutritional character can be attempted, screening methods must be available that allow a large number of samples to be measured with acceptable accuracy and in a short period of time. Plant breeding is to a large extent a matter of chance, and breeding methods have been evolved to maximize the probability of obtaining desirable

gene combinations. It is now recognized by most breeders that the handling of large populations is important in this process. It may be necessary to screen as many as 10 000 individual plant progenies from F_2 and F_3 generations in a major pulse breeding program, and as little as 10 g of seed may be available for testing from each sample. Most breeding programs attempt to grow at least two generations per year to advance as quickly as possible. The nutritional screening of one generation may have to be undertaken in only a few weeks, before the planting of superior genotypes in the next.

In the absence of suitable mass screening methods, the breeder is limited to selecting at a later stage in the program when large quantities of seed are produced, and there are fewer entries to screen. In this case genetic advance, if any, is likely to be slow, but at least it can be ensured that desired standards are maintained.

Genetic Considerations

A breeding program can only be truly efficient if there is some knowledge of the genetic systems involved. Unfortunately there has been relatively little work done on the genetics of nutritional factors in the pulses and there is an urgent need for further study. In the absence of detailed genetic knowledge for a particular species, breeding strategies must be based on existing limited data, and extrapolation from the genetics of other species.

Some of the more important genetic considerations are outlined below.

Genetic variation

Genetic advance can only be achieved if there is genetic variation in the character concerned. Table 1 indicates some of the variation that has been found for protein percentage in the seed of several pulse species. Variation has also been reported in other nutritional factors, e.g., Kelly (1) reported a range in available methionine in dry beans of 0.8–3 mg/g of bean; Porter et al. (2) obtained from 2.15 to 3.16 g of sulfur – amino acids per 16 g N in dry beans; and Yohe et al. (3), in a study of 313 strains of mung beans, found a range in methionine of 5.5–17.8 mg/g of protein. In fact, in all cases in which a large number of genotypes have been studied for a particular nutritional factor, genetic variation has been found. In some cases, however, this reported variation is comparatively small, and the opportunity for genetic advance by selecting within such variation is limited.

Environmentally induced variation

In addition to genetic variation, nutritional factors, in common with most plant characteristics, are subject to variation caused by the environment. The protein content of the seed, for example, may be affected by soil nitrogen levels and the addition of nitrogenous fertilizers or *Rhizobium* bacteria may greatly enhance the percentage protein. Ivanov (4) reported a range of 12.6–31.2% protein in noninoculated and inoculated chick-peas, respectively, within the same cultivar.

Other nutritional factors are also affected by the environment, e.g., Wassimi (5) reported that the soil potassium level influenced cooking quality in lentils, and Bliss (6) reported a significant effect of location on the methionine content of cowpeas.

Because it is only by selecting within genetic variation that genetic advance can be achieved, it is necessary to reduce environmental effects to a minimum. Screening nurseries must thus be planted under uniform conditions, and if possible, in an environment that allows the greatest expression of genetic differences. In a trial to study the influence of the environment on cooking quality in broad beans[1] it was found that at one location all the lines were equally good but at another location, where the average cooking quality of all the lines tested was poorer, considerable genetic variation could be identified.

Genotype × environment interactions

There are several reports in the literature of interactions between the genotype and the environment. Bliss (6), for example, reported significant genotype × location and genotype × location × day length interactions for the protein percentage of cowpea seeds and Sandhu et al. (7) reported significant genotype × year interactions for protein percentage and sulfur content as a percentage of the protein in the seed.

It is desirable that superior cultivars should be nutritionally stable across a range of environments. It is possible that the statistical techniques developed for the analysis of stability and wide adaptation in yield (e.g., the regression analysis of interactions proposed by Finlay and Wilkinson (8)) can usefully be adapted for the study of nutritional factors.

Heritability

Heritability estimates provide a measure of the proportion of the total variance in a character that is of genetic origin. Such estimates are valuable in determining the genetic advance possible through selection. Very few heritability

[1]Personal communication from E.A.E. Ahmed, 1975.

Table 1. Reported range in protein values for selected pulse species.

Species	No. of samples	Range in protein %[a]	Source
Pigeon pea (*Cajanus cajan*)	2262	18.4–28.8	ICRISAT collection
Chick-pea (*Cicer arietinum*)	2667	18.7–28.3	NEC collection[b] ALAD
Lentil (*Lens culinaris*)	1688	23.4–36.4	NEL collection[b] ALAD
Dry bean (*Phaseolus vulgaris*)	4524	17.0–35	Kelly (1)
Broad bean (*Vicia faba*)	511	22.3–37.1	NEB collection[b] ALAD
Cowpea (*Vigna unguiculata*)	118	20.4–28.2	IITA (18)

[a]N × 6.25, dry weight basis.
[b]Germ plasm collections analyzed at the Grain Research Laboratory, Winnipeg, Canada, 1976.

estimates have been reported for nutritional factors in the pulses. Most of these are broad sense heritabilities, based on total genetic variance, rather than the more useful narrow sense heritabilities, which estimate only the additive genetic component as a proportion of the total genetic plus environmental variance.

Major genes

Breeding for the improvement of any character is greatly simplified if major genes can be identified. Such genes, which are simply inherited, can generally be easily transferred by breeding to a desired genetic background. In addition, they frequently have a large effect and tend to be less influenced by environmental variation. As a result, screening procedures often need be less precise when major genes are involved. If the gene has a pleiotropic effect, or is closely linked to a gene conferring an easily identified morphological characteristic, screening is further simplified by selecting on the basis of the pleiotropic or linked character.

In the pulses no major genes have been reported affecting protein quantity or quality, but this should not discourage attempts to find them. In maize, the discovery of the Opaque-2 gene, which confers a higher lysine content in the seed, has enabled breeders to develop "high-lysine" maize cultivars with comparative ease. A few major genes affecting other nutritional factors in the pulses are known. In cowpeas, for example, seeds having a rough testa are easier to dehusk and there is evidence (9) that high vitamin C content in chick-peas is associated with the simply inherited green cotyledon character. In pulses in general, genotypes having a dark seed coat are thought to be nutritionally inferior due to the presence of polyphenols. Seed coat colour is usually a simply inherited character.

Minor genes

The few studies to date of the inheritance of protein quantity and quality in the pulses have all indicated polygenic control of these characters, with additive genetic effects generally being the most important.

Work on peas in Hungary, referred to by Meiners and Litzenberger (10), indicated that in general the protein content of the progeny was intermediate between the parents and was inherited quantitatively. Bond (11) reported that the inheritance of high protein content in broad beans tended to be recessive, but that additive effects were also important.

In view of the existing data, breeding methods that are efficient at combining additive genes are likely to be the most effective for the improvement of protein quantity and quality. Further genetic studies are required, however, to confirm this and to explore the genetic systems involved in the inheritance of other nutritional factors.

Linkage

The useful effects of genetic linkages and pleiotropism have already been referred to in the section "Major genes" above. They can also have undesirable effects, however, and may need to be taken into consideration in a breeding program. A close genetic linkage between a desirable character and an undesirable one may require a large number of crosses to break. Breeders must take care when linkages or pleiotropic effects are suspected, that in breeding for an improvement in one character they are not simultaneously developing a genotype that is poor in another respect. There is some evidence, for example, that good cooking quality in lentils may be associated with poor resistance to weevils. There is also evidence to suggest that resistance to certain pests and diseases may be associated with the presence of polyphenols in the seed coat. In developing genotypes with a low polyphenol content care must be exercised that the pest or disease resistance does not fall to an unacceptable level.

Transgressive segregation

Breeders continually hope for transgressive

segregation (i.e., segregants that fall outside the parental range) in their breeding nurseries. There is evidence that such segregants can be expected for nutritional characteristics, and hence the hope exists of pushing nutritional levels beyond those already identified. Bliss (6) reported finding two F_3 families of dry beans whose means were greater than the mean of the highest parent.

Correlations

Both positive and negative correlations between protein content and seed yield have been reported in the literature. Evans (12) lists correlation coefficients from several sources and her table is reproduced here as Table 2. Bond (11) studied the relationship between yield and protein content in seven trials of field beans and found correlation coefficients ranging from −0.291 to +0.232.

In view of the conflicting evidence on the relationship of protein quantity to yield, there is hope of being able to combine both high protein and high yield in a single genotype. The trials of Leleji et al. (13) demonstrate that at least in their F_2 and F_3 dry bean progenies sufficient variation existed to allow the simultaneous selection of both high yield and high protein.

Other correlations that may be important from a breeder's standpoint have also been reported in the literature; for example, in beans, Rutger (14) reported a negative correlation between seed weight and protein content and Adams (15) reported significant negative correlations between the sulfur-containing amino acids and the nitrogen content of the seed.

Breeding Methods

The choice of a breeding method depends on a number of factors, such as the pollinating system of the plant and the genetics of the characters it is desired to improve.

Most of the pulses are self-pollinated and if out-crossing occurs, it is generally at a low level. Chick-peas, cowpeas, dry beans, and lentils all come in this category. A few species, however, have a much higher level of natural out-crossing, e.g., both broad beans and pigeon peas may reach 40% out-crossing under field conditions. In such cases techniques must be employed to ensure selfing or else breeding methods appropriate for the improvement of cross-pollinated crops may be adapted.

Whatever the pollinating system, a breeder's activities can generally be divided into four separate areas: (*a*) the identification of genetic variation; (*b*) hybridization; (*c*) selection; (*d*) testing and variety release.

Germ Plasm

The initial step in any breeding program is the identification of genetic variation. This is normally done by screening as much of the available germ plasm as possible for the desired characters. The IARCs all maintain inclusive collections for this purpose and germ plasm is made available from these centres to any other breeders. Efforts are currently underway by certain national programs, the IARCs and the FAO Germ Plasm Project to enlarge the existing collections in an attempt to maintain and identify the largest possible range of genes.

If the desired character is not identified within existing germ plasm, a breeder may attempt to introduce genes from related species by interspecific hybridization or to induce mutations through the use of physical or chemical mutagens. These methods are normally only employed, however, as a last resort when the search for genes already present in the species has been unsuccessful.

Once the desired character has been identified, it may be possible to arrive at a superior cultivar merely by selection. In most cases, however, it is

Table 2. The correlation between protein and seed yield (after Evans (12)).

Crop	Correlation coefficient (*r*)	Authority
Soybeans	−0.33	Johnson et al. (20)
	−0.08	
Phaseolus beans	−0.635	Tandon et al. (21)
Phaseolus beans	−0.23	Rutger (14)
	−0.36	
Peas	Low + ve correlation (NS)[a]	Furedi (22)
Phaseolus beans	−0.446	Leleji et al. (13)

[a]NS, not significant.

necessary to transfer the genes to another better-adapted genetic background by a process of hybridization and selection. During this phase of the program many options are open to the breeder and only a few of the more commonly used methods will be outlined in the following sections.

Breeding Methods to Maintain Nutritional Levels

As indicated previously, even if a breeder does not aim to improve nutritional quality, the nutritional level should not be allowed to fall below an acceptable standard. In this case advanced lines must be screened to ensure that varieties of poor quality are not released. Table 3 shows the flow of genetic material in a conventional breeding program such as a pedigree or bulk population system. The generation in which single plant selection or nutritional screening is first carried out may vary from those indicated in the figure, though the basic scheme is common to all.

Following the identification of suitable parents, crosses are made and the F_1s grown out. Selection is rarely carried out in the F_1 generation, except following multiple crosses. Single plant selection may or may not be carried out in the F_2, but frequently single plants are selected in the F_3 generation, and the F_4 is grown as progeny rows. Superior F_4 rows may be bulked for testing in the F_5 generation. It is at this stage that the agronomically superior lines can be screened for nutritional quality, and only those elite lines that have an acceptable nutritional standard should pass to the F_6 yield trial stage. Sufficient quantities of seed are normally available following the F_6 generation to begin biological evaluation.

Breeding Methods for Nutrition Improvement

A breeder may adopt one of several approaches to the nutritional improvement of pulses. In all cases, nutritional improvement should run concurrently with the improvement of other factors such as yield or disease resistance.

Pedigree or bulk breeding

In conventional breeding programs the scheme followed for nutritional improvement follows the same general lines as that given in Table 3. The difference is that at least one of the original parents is chosen as a source of quality genes, and whenever selection is carried out in the segregating generations sequential screening is undertaken. The top 20% of plants or bulks are selected on the basis of yield, agronomic or resistance characteristics, and the best of these are further screened and selected for nutritional quality. It is well known that the heritability of yield is very low in the pulses and sequential selection in the F_2 generation should be on the basis of highly heritable resistance or agronomic characters followed by screening for nutritional factors of high heritability. Selection for yield and nutritional characteristics of low heritability should be reserved for later generations.

Backcross breeding

The backcross system is of particular value when it is desired to transfer genes for a particular nutritional characteristic from a donor gene source to an already elite genetic background. It is most effective for the transfer of major genes, especially if they can be recognized (e.g., in the case of genes for nutritional factors by a morphological pleiotropic effect), before or during flowering.

In conventional backcross breeding program,

Table 3. Outline of a conventional breeding program for the improvement of agronomic characteristics while maintaining the nutritional status.

Season	Generation (parent/seed)	Operation
1	Parents/F_1	Hybridization
2	F_1/F_2	No selection
3	F_2/F_3	Single plant selection and (or) bulking
4	F_3/F_4	Single plant selection
5	F_4/F_5	Bulking of best progeny rows
6	F_5/F_6	Testing of bulks. Superior bulks screened for nutritional quality
7	F_6/F_7	Further yield testing and biological evaluation

Table 4. Outline of a hypothetical backcross breeding program to transfer major genes for high protein to an agronomically elite genetic background. Only three backcrosses are shown.

Season	Generation	Remarks
1	$P_1 \times P_2$	P_1 = recurrent, elite parent; P_2 = source of high protein gene
2	$F_1 \times P_1$	First backcross to recurrent parent
3	B_1	Grow out B_1 generation, select single plants on the basis of high protein
4	$B_2 \times P_1$	Second backcross
5	B_1	F_1 generation following second backcross; single plants selected on basis of yield and high protein
6	$B_2 \times P_1$	Third backcross
7	B_1	F_1 generation following third backcross; single plants selected on basis of yield and high protein

backcrosses are normally made between the B_1 (the generation following the backcross) and the recurrent parent. In attempting to transfer genes for nutritional quality in pulses, however, backcrosses are made between the B_2 generation and the recurrent parent, to allow selection for nutritional quality in the B_1/B_2 seed.

Table 4 gives an outline of a theoretical backcross breeding program for the transfer of genes for high protein to an agronomically elite genetic background. Only three backcrosses are shown in the table but in practice up to six or seven may be made.

Population improvement

Population breeding methods were originally developed for the improvement of cross-pollinated species, but their use has since extended to self-pollinated crops that have a high level of out-crossing or that are easy to pollinate by hand. The methods are particularly suited to the improvement of characters controlled by additive genes because a large number of parents can be used in the constitution of a population. In addition, because of the repeated cycles of hybridization and selection, a greater opportunity is provided for breaking up genetic linkages.

The basic scheme for population improvement in pulses is shown in Table 5, though many modifications are possible. The first step in the scheme is the setting up of the original population. This is done by crossing between the parents, either at random or in a controlled manner, for several generations to ensure a thorough mixing of the genes. Random crossing may be achieved through the use of pollinating insects in certain pulses such as pigeon peas and broad beans. The scheme is also possible if large numbers of crosses can be made by hand, either in crops that are easy to cross, or through the use of genetic male sterility. The identification of a reliable gametocide would allow the method to be used in those crops in which male sterility has not yet been discovered.

Following the constitution of the original population, the S_0 generation (tentative F_1) is grown out and single plants can be selected. The progeny of the selections are grown out in the S_1 (first selfed) generation and selection is carried out on a row basis. Superior rows in the S_1 are tested in the S_2 generation at several locations and the superior entries recombined the following generation. The cycle is then repeated and each cycle should result in an improvement of the population. At any stage superior plants can be selected and handled by normal pedigree or bulk population systems.

The method outlined above is based on S_2 testing; however, Rachie and Gardner (16) outlined several other population breeding methods that can be used in partially out-crossing species, and other methods, e.g., the diallel selective mating scheme (17), have been developed for use in self-pollinated crops. S_2 family testing requires four generations per cycle, which, in short duration crops like some of the *Vigna* and *Phaseolus* species, may be undertaken in a single year. More often 18 mo or 2 yr are required per cycle. Modifications such as S_1 family testing, half-sib family testing, and mass selection, requiring three, two, and one generations per cycle, respectively, may be adapted for pulse improvement in certain cases, though with

Table 5. Outline of a population breeding program using an S_2 testing procedure.

Step	Operation	
1	Constitution of original population by random or controlled crossing between parents for several generations	
2	The S_0 generation is grown out; single plants are selected for desired characters	One cycle
3	The S_1 (first selfed) generation is grown out in progeny rows; selection is carried out on a row basis	
4	The best rows from the S_1 generation are tested in the S_2 at several locations	
5	The best entries in the S_2 trials are recombined by random or controlled crossing	

correspondingly reduced precision and efficiencies.

In a breeding program based on population improvement methods, the mainstream of the program should be devoted to the improvement and stabilization of yield and other agronomic characters. Separate subpopulations can be established for developing other characters such as disease resistance and nutritional factors. The selection pressure applied to the subpopulation will be for a single character only, and when sufficient advance has been made in the subpopulation, the character can be transferred to the mainstream population through an intermediate back-up population. This process is shown in Fig. 1.

Future Trends in Nutritional Improvement

Breeding for nutritional improvement in the pulses is still in its infancy and future research will undoubtedly enable breeders to handle their programs more effectively. At present, progress in nutritional improvement is limited by (*a*) the paucity of genetic knowledge, (*b*) the lack of suitable screening methods for many nutritional factors, and (*c*) the immediate need for emphasis on yield and agronomic and resistance characteristics.

The discovery of major genes for nutritional factors could revolutionize breeding for nutritional quality, especially if such genes are easily identified phenotypically. The search for male sterility in all pulses is likely to succeed and would enable many more crosses to be made. Likewise the development of gametocides would greatly simplify the hybridization process.

Techniques for crossing between species could play an important role in future pulse improvement programs and research is needed to identify closely related wild species having genes for superior nutritional quality. In a recent screening of eight lines of *Vicia narbonensis* (a species closely related to *V. faba*) one was found having over 33% protein in the seed. Some success has recently been attained in wide crossing among *Phaseolus* species in which lima beans (*P. lunatus*) were crossed with *P. pedicellatus* and *P. ritensis*, and *P. vulgaris* was crossed with *P. ritensis* (18). A bridge may thus be provided between *P. vulgaris* and *P. lunatus* that could result in the development of a broader range of genetic variability in both species. The initial results of crossing between lentils (*Lens culinaris*) and *L. orientalis* at ALAD also look promising.

Note: High protein lines have been identified at ICRISAT in derivatives of crosses between *Cajanus cajan* and *Atylosia* sp. F_6 lines from crosses with *Atylosia scarabaeoides* and *Atylosia sericea* have given slightly over 30% protein, while within the *Cajanus* germ plasm the highest reading has been 28.8%.

Success in breeding for nutritional improvement may also be achieved through screening for physiological processes. As more becomes known about the ways in which a plant manufactures its protein, for example, so the possibility exists of screening for greater efficiency. Sprague (19) reported on a screening process to determine a plant's capacity to reduce nitrate to nitrite, one of the steps involved in the synthesis of protein. By combining genes conferring efficiency for each step of the process, it is con-

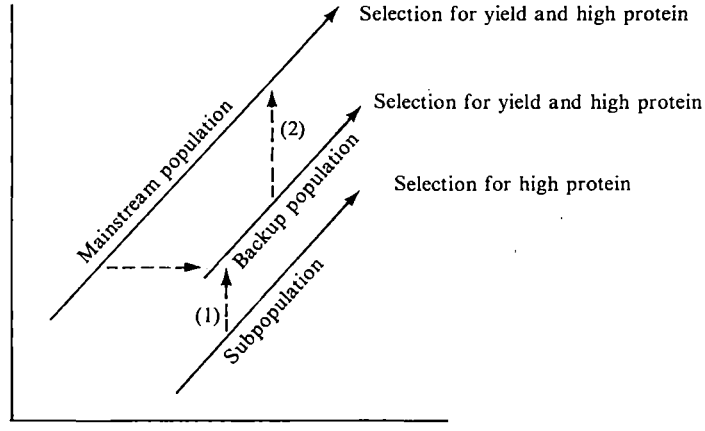


Fig. 1. Outline of a population breeding program for the improvement of yield and protein content. (1) Superior plants from mainstream populations are combined with high protein selections to form the backup population. (2) Plants combining high yield with high protein are fed into the mainstream population.

ceivable that a genotype could be developed having a very good overall efficiency for producing protein.

As better screening techniques are developed, it will be possible to handle greater quantities of material with greater accuracy. The ultimate screening method will be nondestructive measurement of a single seed, as is already possible in measuring oil content by nuclear magnetic resonance.

References

1. Kelly, J.F. 1973. Discussant in nutritional importance of food legumes by breeding. New York, U.S., Protein Advisory Group/Food and Agriculture Organization of the United Nations, 159–161.
2. Porter, W.M., Maner, J.H., Axtell, J.D., and Keim, W.F. 1974. Evaluation of the nutritive quality of grain legumes by an analysis for total sulfur. Crop Sci. (U.S.), 14, 652–654.
3. Yohe, J.M., Swindell, R.E., Watt, E.E., Bashandi, M.M.H., Sechler, D.T., and Poehlman, J.M. 1972. In Evaluation of mungbean strains at Columbia, Missouri in 1971. Miscellaneous publication No. 72-9. Columbia, Mo. U.S., Department of Agronomy, Missouri Agricultural Experimentation Station.
4. Ivanov, N.N. 1933. The causes of chemical variability in the seeds of chickpea in different geographical areas. Quoted in Van der Maesen 1972. *Cicer* L.A. monograph of the genius. Wageningen, Agriculture University Communication.
5. Wassimi, N. 1976. Effect of plant nutrition on the cooking quality of lentils. M.Sc. thesis, American University of Beirut, Lebanon.
6. Bliss, F.A. 1973. Cow peas in Nigeria. In Nutritional importance of food legumes by breeding. New York, U.S., Protein Advisory Group/Food and Agriculture Organization of the United Nations, 151–158.
7. Sandhu, S.S., Keim, W.F., Hodges, H.F., and Nyquist, W.E. 1968. Inheritance of protein and sulphur content in chickpeas. In Agronomy Abstract, Madison, Wisc., U.S.
8. Finlay, K.W., and Wilkinson, G.N. 1963. The analysis of adaptation in a plant breeding programme. Aust. J. Agric. Res. (Australia), 14, 472–754.
9. Chandra, S., and Arora, S.K. 1968. An estimation of protein, ascorbic acid and mineral matter content in some indigenous and exotic varieties of gram (*Cicer arietinum*)(L). Curr. Sci. (India), 37(8), 237–238.
10. Meiners, J.P., and Litzenberger, S.C. 1973. Breeding for nutritional improvement. In Nutritional importance of food legumes by breeding. New York, U.S., Protein Advisory Group/Food and Agriculture Organization of the United Nations, 131–141.
11. Bond, D.A. 1970. The development of field beans as a crop in Britain. Proc. Nutr. Soc. (England), 29, 74–79.
12. Evans, A.M. 1973. Genetic improvement of *Phaseolus vulgaris*. In Nutritional importance of food legumes by breeding. New York, U.S., Protein Advisory Group/Food and Agriculture Organization of the United Nations, 107–115.
13. Leleji, O.I., Dickson, M.H., Crowder, L.V., and Bourke, J.B. 1972. Inheritance of crude protein percentage and its correlation with seed yield in beans, *Phaseolus vulgaris* L. Crop Sci. (U.S.), 12, 168–171.

14. Rutger, J.N. 1970. Variation in protein content and its relation to other characters in beans, *Phaseolus vulgaris*. Vol. 10. In Report on dry bean research conference, Davis, Calif., U.S., 59–69.
15. Adams, M.W. 1973. On the quest for quality in the field bean. In Nutritional importance of food legumes by breeding. New York, U.S., Protein Advisory Group/Food and Agriculture Organization of the United Nations, 143–149.
16. Rachie, K.O., and Gardner, C.O. 1976. Increasing efficiency in breeding partially outcrossing grain legumes. In International Workshop on Grain Legumes, ICRISAT, Hyderabad, India. 13–16 Jan 1975, 285–302.
17. Jensen, N.F. 1970. A diallel selective mating system for cereal breeding. Crop. Sci. (U.S.), 10, 629–635.
18. International Institute of Tropical Agriculture. 1976. International Institute of Tropical Agriculture, 1975 Annual Report, Ibadadan, Nigeria, 75–125.
19. Sprague, G.F. 1974. New way to evaluate herbicides. In Crops and soils, Jan 1974. 15–16.
20. Johnson, H.W., Robinson, H.F., and Comstock, R.E. 1955. Genotypic and phenotypic correlations in soybeans and their implications in selection. Agr. J. 47, 477–483.
21. Tandon, O.B., Bressani, R., Scrimshaw, N.S., and LeBeau, F. 1957. Nutrients in Central American beans. J. Agric. Food Chem. (U.S.), 5, 137–142.
22. Furedi, J. 1970. Possibilities of hybridization to increase protein improvement in pea. Hung. Acad. Sci. Agr. Dept. Proc. (Hungary), 29, 363–376.

Tentative Nutritional Objectives in the Major Food Crops for Plant Breeders

R. Bressani and L.G. Elías

In recent years, renewed attention by agricultural scientists has been given to the increased production of cereal grains and food legumes, because these basic foods can provide the basic energy and protein needs of populations living in the developing countries. To achieve increased productivity, plant breeders and other agricultural scientists are maximizing the efficiency of the plant to utilize energy, carbon dioxide, water, and soil nutrients; attention is also being given to a more efficient supply of limiting soil nutrients and to biological processes dealing with a more efficient control of plant diseases and pests.

The full meaning of productivity is not complete, however, if it stops with increased production per unit of arable land. Productivity (kilograms per hectare) must be viewed as the efficiency with which the nutrients in the food best meet the needs of the population, with minimum of waste. As expressed by the equation in Table 1, more efficient use of available arable land and of other factors in food production is concerned with production/hectare when corrected by the nutritive value and a technological value.

The basis for selection of food crops must, therefore, be based on production/hectare as the first component of productivity, modified by the nutritional quality, and finally by a technological index. The nutritional quality factor refers mainly to protein. The protein quality factor can be determined either by improvement of the nutritional composition of the food item by itself, or on the basis of the pattern of total food consumption of which the food item is commonly part. From the practical point of view the second approach appears sounder as well as easier to attain. The technological index refers to the attributes the food must have to be acceptable to both the consumer and the food processor.

Although increased production must continue to be the most important objective, nutritional considerations are also important to improve the efficiency of utilization of the food produced. Programs in this particular research area appear to have been impaired by the lack of clear objectives and recommendations to be followed by plant breeders; or in some instances, goals proposed by nutritionists are too ambitious and therefore difficult to attain.

Table 1. Basis for selection of food crops.

Productivity = Yield (kg/ha) × Nutritive value × Technological value
Nutritive value of the food itself or as component of a diet.
Expressed as utilizable protein or in terms of percentage of protein-calorie of total calories.
Technological value (includes all functional characteristics) expressed as percentage of standard.

Table 2. Range in protein content in four species of legume foods.

	No. samples	Protein content, %			Reference
		Low	High	Average	
Phaseolus vulgaris	21	17.7	31.6	23.8	INCAP/ICTA, unpublished data
Vigna sinensis	21	22.9	34.6	29.3	(1)
Cajanus cajan	85	18.1	22.3	21.0	(2)
Cicer arietinum	29	21.5	25.1	23.5	(2)

Furthermore, rapid chemical and biological methods have not been developed to help geneticists to proceed faster toward their objective.

The primary purpose of this paper is to list a series of considerations on which plant breeders can base their intervention to improve the nutritive value of the main food commodities. It is also meant to stimulate research to enable more precise determination of the limiting nutrients in the various food crops.

Basic Nutritional Information Required

The objective of plant breeding in terms of introducing superior nutritional characteristics into food crops must be based on at least two kinds of considerations: those related to the variability in the nutrient content reported for each particular crop, and those pertaining to the patterns of consumption of each specific food in relation to other foods.

Variability in nutrient content

Food composition tables indicate that, for a variety of cereal grains and food legumes, there is a relatively large variation in the content of some nutrients, particularly vitamins, with less variation of the main components such as protein.

Table 2 summarizes the variation in protein content of four species of legume foods. In *Phaseolus* and *Vigna* the variation among 21 cultivars is relatively large; however, in *Cajanus* and *Cicer*, protein content appears to be less variable (1, 2 and INCAP/TCTA, unpublished data).

With respect to the amino acid content of protein of cereal grains and legume foods, information available suggests some variation, which often has been indicated to be either of genetic or of environmental origin. The knowledge that such variation exists was demonstrated by the increased concentration in lysine and tryptophan in Opaque-2 corn (3), and high lysine content in barley (4) and sorghum (5). Variation in amino acid content in legume foods is also found, as shown by values in five species for five essential amino acids (Table 3). Such variation is probably due to the genetic composition of the legume, although environmental effects should not be disregarded (6).

Food consumption patterns

Dietary surveys and similar sources of data indicate that the diet of the majority of the population in developing countries is based on a cereal grain and a legume food. For other groups in the same areas, starch roots, tubers, and legume grains constitute the bulk of the diet. Because of this, cereal grains will continue to provide a significant amount of the energy needs as well as a considerable amount of protein; however, legume grains will continue to represent the main source of supplementary protein in these diets. A representative example (Table 4) shows the composition of diets consumed by preschool children in a rural area of Guatemala, and Table 5 summarizes the proximate analysis of these diets (7).

On a dry-weight basis, cereal grains in diets amounts to around 80% of the total and legume grains to about 10%, with the difference being made up by other foods such as vegetables, sugar,

Table 3. Reported range in levels of limiting amino acids of legume foods.

Amino acid	*Phaseolus vulgaris*	*Glycine max*	*Arachis hypogaea*	*Vigna sinensis*	*Cicer arietinum*
Methionine	28–131	53–114	33–100	50–119	34–106
Cystine	21–108	51–114	11–106	48–106	50– 94
Tryptophan	32–101	75– 88	45– 90	66– 70	25– 94
Valine	213–388	250–375	142–307	250–325	213–356
Threonine	192–356	200–285	116–207	178–300	219–263

Table 4. Amounts and ingredients in the diets consumed by preschool children in a rural area of Guatemala.

Food items	Intake g/day	Intake %
Tortilla (maize)	103.5	31.9
Bread	19.5	6.0
Peas	47.9	14.7
Coffee	2.6	0.8
Sugar	28.9	8.9
Meat broth	26.4	8.2
Meat	4.8	1.5
Eggs	7.8	2.4
Vegetables	21.2	6.5
Rice and pasta	16.1	4.9
Fruits	13.0	4.0
Banana	16.4	5.1
Potato	4.3	1.3
Bean broth	12.5	3.8
	324.9	100.0

coffee, and fruits. A large part of the protein is contributed therefore by cereals and legumes. These diets are consequently low in total protein and deficient in the amino acids limiting the protein quality of the cereal grain (8).

Nutritional characteristics of cereal grains and legume foods

Cereal grains are nutritionally characterized by low levels of total protein and deficiencies in essential amino acids, among which lysine is the most important. They are relatively good sources of sulfur-containing amino acids. On the other hand, legume grains contain twice as much protein as cereal grains. This protein is a rich source of lysine although relatively low in total sulfur-containing amino acids. Starchy tubers have various nutritional disadvantages, namely high water and low protein content. Furthermore, true protein content is about half of the total crude protein and is deficient in sulfur-containing amino acids. Even though the amount of legume-grain in the diet is small, the protein quality of the combination cereal grain - legume food is increased in some diets up to 60% over that of the cereal grain alone (Table 6) (9). Therefore, if the objective is to improve the nutritional quality of the diet, it is important to realize that food items are not consumed alone but rather in a mixed diet. This is important in establishing nutritional goals in these two main basic food crops.

Approach to Arriving at Nutritional Standards

In establishing nutritional standards for the basic food crops, one method is to improve the quality of the food by itself, and for such a purpose the reference standard could be the FAO/WHO amino acid scoring pattern (10). This approach seems difficult to attain and probably is unnecessary as only a few proteins meet such a pattern. The second approach is to set up nutritional goals for the basic food as part of the food consumption system in which it is used. This approach is probably easier to achieve, since the levels set are not as high as those in the scoring pattern, but are sufficient to complement the level in other foods in the system. This is, therefore, the approach indicated below.

Increase the protein concentration in the cereal grain and legume food

Table 7 shows the effect of increasing the protein quality and quantity of maize and beans on the nutritional value of a diet made of the two foods. The results in terms of weight gain, protein efficiency ratio (PER), and utilizable protein show that the protein for common maize in the diet is improved by the addition of lysine and tryptophan. An improvement, although not as high, is also seen when the maize used had a higher protein content, particularly in utilizable protein. More important however, is that with maize of a higher protein content in the diet, the amino acids added caused more than a twofold increase in weight gain, PER, and utilizable protein. These results indicate a greater beneficial effect with a higher protein maize with higher levels of lysine and tryptophan.

Similar results were also obtained when *using twice the level* of beans in the maize-bean diet, which provided a *higher protein intake*. The effect of increased content of beans was observed with both the normal or the higher protein maize. On the other hand, no improvement is observed

Table 5. Proximate analysis of rural diets consumed by preschool children in Guatemala.

	Santa María Cauqué, diet	
	Day, g	%
Moisture	210.2	64.7
Dry matter	114.7	35.3
Protein	14.0	4.3
Fat	2.9	0.9
Crude fiber	2.9	0.9
Ash	2.6	0.8
Gross energy	455.0	140.0
% protein adequacy, children 12–13 mo	82.0	
% caloric adequacy, children 12–13 mo	76.0	

Table 6. Protein quality and utilizable protein of cereal grains supplemented with beans (9).

	Protein	PER[a]	Utilizable protein, g%
Maize, 100%	8.5	0.87	2.41
Maize, 90% Beans, 10%	10.3	1.40	4.10
Sorghum, 100%	7.7	0.88	2.23
Sorghum, 90% Beans, 10%	8.6	1.39	3.43
Wheat, 100%	11.0	1.05	4.26
Wheat, 90% Beans, 10%	12.0	1.73	5.94
Rice, 100%	6.9	2.15	4.01
Rice, 90% Beans, 10%	7.9	2.32	4.96

[a]PER, protein efficiency ratio.

Table 7. Effect of the improvement in quality and quantity of protein on the nutritive value of the basal rural diet consumed by preschool children in Guatemala. (Experiment with growing rats.)

Change in basal diet[a]	Protein in diet, %	Avg wt gain, g/28 days	PER[b]	Relative nutritive value, %	Utilizable protein, %
Maize — A (normal protein) (8.3%)	9.7	45	1.67	44.7	4.33
Maize — B (high in protein) (13%)	12.4	56	1.52	40.7	5.05
Maize — A + Lys[c] + Try[d]	10.0	81	2.49	66.7	6.67
Maize — B + Lys + Try	12.6	119	2.45	65.6	8.26
Bean protein increase (2 ×)	10.5	72	2.12	56.8	5.96
Maize — B + Lys + Try + Bean protein increase (2 ×)	11.9	100	2.34	62.7	7.45
Bean + methionine	9.0	41	1.65	44.2	3.98
Bean protein increase (2 ×) + methionine	10.8	70	1.98	53.0	5.72

[a]Maize, 72.4% + bean, 8.10%.
[b]PER, protein efficiency ratio.
[c]Lys, lysine.
[d]Try, tryptophan.

Table 8. Effect of changing protein content in basic food, keeping intake constant, on total protein intake for preschool children.

	Intake, g/100 g diet	Present case		Protein increase in maize		Protein increase in maize and beans	
		Protein, %	Total protein, g	Protein, %	Total protein, g	Protein, %	Total protein, g
Maize	90	9	8.1	14	12.6	14	12.6
Beans	10	23	2.3	23	2.3	28	2.8
Total	100	—	10.4	—	14.9	—	15.4
Intake of solid food by children (avg): 115 g Avg wt of children: 14 kg Protein requirement: 1.25 g/kg per day							
Total protein intake: g/115 g			12 g		17.1		17.7
Intake/kg			0.86		1.22		1.26
Adequacy, %[a]			68.8		97.6		100.8

[a]Assuming biological value of 100%.

when a higher methionine, normal protein bean is used, or a higher methionine with high protein bean. An advantage to high protein content in both foods is that the protein requirements can be met, as is indicated in Table 8, using as an example the diet consumed by the preschool children in Guatemala (11).

Increase the intake of the legume grain at the expense of cereal grain

Some of the evidence on this procedure, with use of different mixtures of normal maize and black beans as well as Opaque-2 maize and black beans, is shown in Fig. 1. The results clearly show the nutritional benefits obtained by the isonitrogenous replacement of common maize by black bean nitrogen up to 50:50 protein ratio distribution level. Obviously, in the case of Opaque-2 maize, the higher response obtained at all levels of protein combination is the effect of an improvement in the protein quality of the different mixtures. From a practical point of view, the increased intake of legume foods at the expense of cereal grain results in a diet of a higher protein quantity and quality, due to the mutual complementation of their proteins up to the point indicated in the curves. Experimental evidence has also demonstrated that to the left of the maximum point, methionine is more deficient as the protein of the diet contains more bean protein, whereas to the right of the maximum point, lysine becomes the most limiting amino acid, as the protein of the diet contains more corn protein. At the maximum point the diet is deficient in both amino acids. The results illustrated with these curves are clearly related to the amino acid values of the two basic foods studied (12).

Present intake of corn and beans in Guatemala is shown by the bar to the right of the optimum combination. As indicated, this diet has a lower protein quality than the best mixture, and is deficient in lysine. Its quality can be improved to some extent by increasing beans and to a larger degree by adding amino acids or protein supplements.

The amounts added serve to suggest what should be the nutritional standards for the limiting amino acids in the two foods. In this case, and to achieve the quality line of Opaque-2 corn, the amino acid to be increased in the two foods would be lysine contributed by the two foods (13).

By following the previous technique, similar improvement is obtained when rice is replaced by cooked black beans, as shown in Fig. 2. However, in this case, the maximum point is attained when rice provides 80% of the protein and beans 20%. The increase in protein quality from the 100% rice to the 80% rice diet was due to the lysine and threonine contribution from 20% beans, while rice provided beans with methionine. These effects can be seen from the amino acid content of the two proteins studied, as shown in the same graph.

Fig. 2 shows on the right the effect of adding higher amounts of their limiting amino acids to these two basic foods. The results indicated that even though a mixture of rice and beans has a protein quality higher than each component alone, the individual components as well as any of the mixtures may be improved if their respective deficient amino acids are present in higher amounts (14). The same type of result is obtained with cowpea and corn as well as with other legume foods (15–17) as shown in Fig. 3, which is a simplified version of individual studies with the two-component system. The responses observed indicate the benefits of improved protein quality that result when Opaque-2 corn replaces common corn in mixtures with common beans. Likewise, it shows the differences in protein quality when common corn is mixed with three species of legume foods. Best performance is obtained from soybeans, followed by cowpeas, and last by common beans. Much could therefore be achieved nutritionally if common beans had the nutritional attributes of cowpeas, or better, of soybeans. Table 9 shows some of the important essential amino acids in the three legume foods, which suggests that the better performance of soybean and cowpea than common beans, is due to their higher sulfur amino acid and tryptophan content.

Table 9. Some essential amino acids in common beans, cowpeas, and soybeans.

Amino acid	Common beans	Cowpeas	Soybeans
Total sulfur-containing amino acids	125	177	195
Tryptophan	58	60	86
Lysine	464	407	395
Threonine	271	246	246

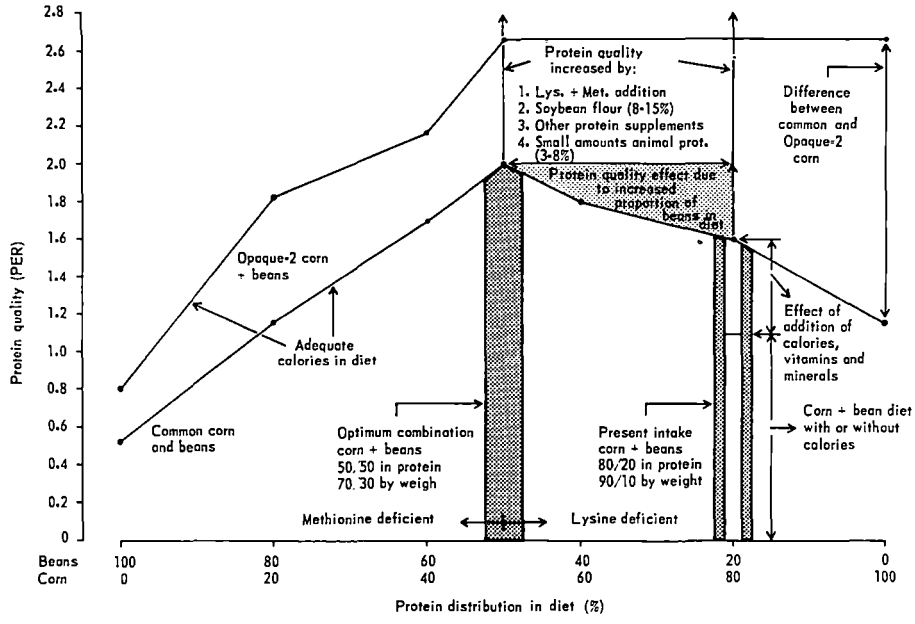
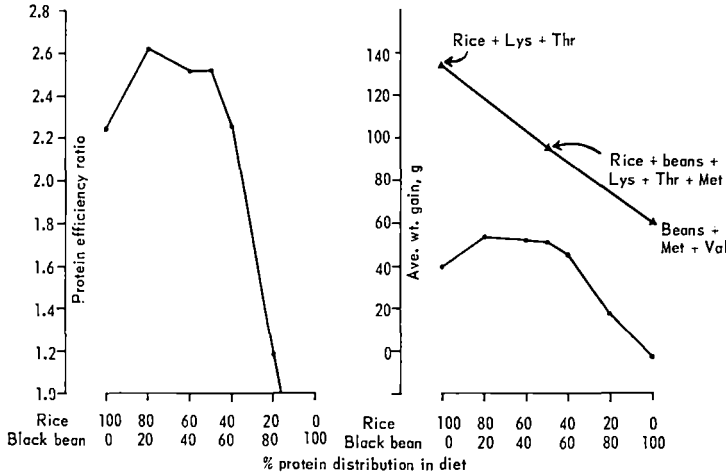


Fig. 1. Results of increasing the intake of the legume grain at the expense of cereal grain, using mixtures of normal maize and black beans as well as Opaque-2 maize and black beans.



	Lys	Met	Thr
	mg·g N		
Rice	235	188	233
Black beans	464	125	271

Fig. 2. Protein complementation between rice and cooked black beans.

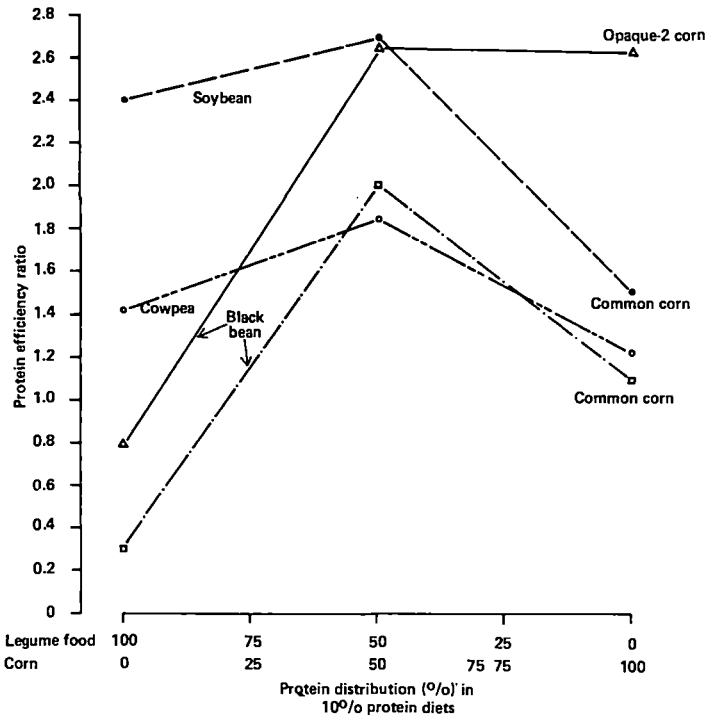


Fig. 3. Mixtures of maximum protein quality between a legume food and corn.

Improve the essential amino acid pattern of the cereal grain and(or) of the legume food

Table 10 shows some of the implications of this approach on the nutritive value of a maize–bean diet. The addition of lysine and tryptophan to maize resulted in a significant improvement in the protein quality of the diet; however, the addition of methionine to beans, in the absence or presence of added lysine and tryptophan to maize, does not improve the protein quality of the diet. These findings suggest that in cereal grains – legume diets, there is little advantage in raising the methionine content of beans. Furthermore, this table indicates that lysine and tryptophan are the most limiting amino acids in the maize–bean diet (18, 19).

Additional evidence is seen in the results summarized in Table 11. The addition of lysine and tryptophan to the common maize induced a significant increment in the nitrogen retention, when compared with that obtained with the common maize without supplement. This increment in the nitrogen retention was due to a lower excretion of urinary nitrogen, the fecal nitrogen being similar in both diets. When Opaque-2 maize was used in the diet instead of common maize, nitrogen retention was similar to that obtained with the basal diet supplemented with lysine and tryptophan. These results clearly justify the efforts that have been made to increase the lysine and tryptophan content in common

Table 10. Effect of the addition of lysine and tryptophan to maize or methionine to beans on the nutritive value of a maize–bean diet.[a] (Experiment with growing rats.)

Treatments	Avg wt[b] gain, g/38 days	PER[c]
Maize Beans	69	2.11
Maize + Lys + Try[d] Beans	103	2.64
Maize Beans + methionine[e]	66	1.93
Maize + Lys + Try Beans + methionine	108	2.69

[a] Maize, 72.4% + beans, 8.10%.
[b] Average-initial weight: 44g.
[c] PER, protein efficiency ratio.
[d] L-Lys HCl, 0.30%, DL-Tyr, 0.10%.
[e] DL-Methionine, 0.30%.

Table 11. Nitrogen retention of young dogs fed a maize–beans diet with and without supplements.[a]

Basal diet	Nitrogen balance, mg/kg per day				
	Intake	Fecal	Urine	Absorbed	Retained
Common maize	300	152	206	247	41
Common maize + lysine + tryptophan	374	156	143	218	75
Common maize	357	157	165	200	35
Opaque-2 maize	407	165	127	242	115

[a]Average of six dogs and of three nitrogen balances of 4 days each, by treatment. Protein intake: 2.5 g/kg per day.

maize, or the lysine content in other cereal grains (20).

The special problem of cassava-bean diets

Large groups of people consume diets based on roots and beans. Root crops such as cassava have little protein, and in such diets the total protein ingested is derived from beans. This food should therefore have a higher protein content and quality for such a food system.

As shown in Table 12, to obtain positive weight gain on a cassava diet 26% beans of higher methionine content was needed. An intake of 39% beans induced some weight gain, but less than observed with 26% beans plus methionine (21).

Proposed Protein and Amino Acid Levels in Some Basic Foods

The protein, lysine, tryptophan, and sulfur-containing amino acid content of various cereal grains and legume foods listed in Table 13 was calculated from the kind of studies briefly discussed in the previous sections. For protein the figures represent maximum desirable values, and for the essential amino acids the various food items should have not less than the values shown. The figures are not to be taken without question, and the authors recognize that there are many limitations to the nutritional methodology employed to reach such calculations. It is also recognized that more precise values may be

Table 12. Effect of adding methionine on the nutritive value of various cassava–beans mixtures.

Dietary treatment	% protein in diet	Wt changes, g/28 days	PER	Cassava–bean ratio
Cassava (100)[a]	1.9	-16.0	—	—
Cassava (87)[a] Bean (13)	4.3	-10.0	—	6.7
Cassava (87)[a] Bean (13) + methionine[b]	4.1	- 7.0	—	6.7
Cassava (74)[a] Bean (26)	6.4	- 6.4	—	2.8
Cassava (74)[a] Bean (26) + methionine[b]	6.7	+29.0	1.78	2.8
Cassava (61)[a] Bean (39)	8.8	+11.0	0.66	1.56
Cassava (61)[a] Bean (39) + methionine[b]	9.0	+67.0	2.42	1.56

[a]Figures in parentheses refer to amounts, in grams, in the diet.
[b]Amount added: 0.03 g of DL-methionine per gram of bean protein.

obtained by further research and that they must be subjected to biological confirmation. It is hoped that this type of approach to the problem of improving the protein quality of the main staple foods will stimulate more research in this area.

Table 13. Proposed nutrient levels in various cereal and legume grains of common consumption in developing countries.

Basic food	Protein	Lysine, g/16 g N	Tryptophan, g/16 g N	Total sulfur amino acids, g/16 g N
Maize	13–14	3.6–3.8	0.80–0.90	3.2–3.4
Sorghum	14–15	3.6–3.8	0.80–0.90	3.2–3.4
Rice	8–10	3.5–3.6	0.80–0.90	3.2–3.4
Beans[a]	28–30	6.3–6.4	1.10–1.20	2.3–2.4
Beans[b]	28–30	6.3–6.4	1.10–1.20	3.2–3.4
Cowpea[a]	28–30	6.3–6.4	1.10–1.20	2.3–2.4
Cowpea[b]	28–30	6.3–6.4	1.10–1.20	3.2–3.4
Pigeon pea[a]	28–30	6.3–6.4	1.10–1.20	3.2–3.4

[a]When supplemented to cereal grains.
[b]When supplemented to cassava-based diet.

References

1. Boulter, D., Evans, I.M., Thompson, A., and Yarwood, A. 1973. The amino acid composition of *Vigna unguiculata* (cowpea) meal in relation to nutrition. In Milner, B., ed., Nutritional improvement of food legumes by breeding: proceedings of the symposium of PAG, New York, U.S., 3–5 July 1972. New York, U.S., Protein Advisory Group, United Nations, 205–215.
2. Hulse, J.H. 1975. Problems of nutritional quality of pigeon pea and chickpea and prospects of research. In International workshop on grain legumes, 13–16 Jan 1975. Begumpet, Hyderabad 500016 (AP), India, International Crops Research Institute for the Semi-Arid Tropics, ICRISAT 1-11-256.
3. Mertz, E.T., Bates, L.S., and Nelson, O.E. 1964. Mutant gene that changes protein composition and increases lysine content of maize endosperm. Science (U.S.), 145, 279–280.
4. Munck, L., Karlsson, K.E., Hagberg, A., and Eggum, B.O. 1970. Gene for improved nutritional value in barley seed protein. Science (U.S.), 168, 985–987.
5. Singh, R., and Axtell, J.D. 1973. Biological value of high lysine (he) and sugary (su) mutants. In Axtell, T.D., ed., Inheritance and improvement of protein quality and content in sorghum. Report No. 10, Purdue University, Lafayette, U.S.
6. Kelly, J.F. 1973. Increasing protein quantity and quality. In Milner, M., ed., Nutritional improvement of food legumes by breeding: proceedings of the symposium on PAG, New York, U.S., 3–5 July 1972. New York, U.S., Protein Advisory Group, United Nations, 179–184.
7. Murillo, B., Cabezas, M.T., and Bressani, R. 1974. Influencia de densidad calórica sôbre utilización de proteína en dietas a base de maíz y frijol. Arch. Latinoam. Nutr. (Venezuela), 24, 223–241.
8. Elías, L.G., and Bressani, R. 1971. Improvement of the protein quality of corn–bean diets by the use of fortified corn or Opaque-2 corn. In Western Hemisphere nutrition congress III. Bal Harbour, Miami, Florida, U.S., August 30/September 2, 1971.
9. Bressani, R., Flores, M., and Elías, L.G. 1973. Acceptability and value of food legumes in the human diet. In Potentials of field beans and other food legumes in Latin America. Centro Internacional de Agricultura Tropical, Series Seminar 2E, Cali, Colombia. 17–48.
10. Food and Agriculture Organization of the United Nations/World Health Organization. 1973. Energy and protein requirements. WHO Technical Report Series No. 522.
11. Bressani, R., Elías, L.G., and Flores, M. 1971. Basic information required before enrichment and/or fortification program. In Workshop on food enrichment and fortification program, 7–9 June 1971, Washington, D.C.
12. Bressani, R., Valiente, A.T., and Tejada, C. 1967. All-vegetable protein mixtures for human feeding. VI. The value of combinations of lime-treated corn and cooked black beans. J. Food Sci. (U.S.), 27, 394–400.
13. Bressani, R., and Elías, L.G. 1969. Studies on the use of Opaque-2 corn in vegetable protein rich foods. J. Agr. Food Chem. (U.S.), 17, 659–662.
14. Bressani, R., and Valiente, A.T. 1962. All-vegetable protein mixtures for human feeding. VII. Protein complementation between polished rice and cooked black beans. J. Food Sci. (U.S.), 27, 401–406.
15. Bressani, R., and Scrimshaw, N.S. 1961. The development of INCAP vegetable mixtures. I. Basic Animal Studies. In Meeting protein needs of

infants and preschool children: Proceedings of the International Conference, Washington, D.C., 1960, and NAS/NRC Publication 843, Washington, D.C., 35–48.
16. Bressani, R., Murillo, B., and Elías, L.G. 1974. Whole soybeans as a means of increasing protein and calories in maize-based diets. J. Food Sci. (U.S.), 39, 577–580.
17. Bressani, R. 1975. Nutritional contribution of soy protein to food systems. J. Am. Oil Chem. Soc. (U.S.), 52, 254A–262A.
18. Elías, L.G., and Bressani, R. 1970. Factores nutritivos limitantes en dietas rurales de Centro América. II. Reunión de la Sociedad Latinoamericana de Nutricion (SLAN) llevada a a cabo en Vina del Mar, Chile. Diciembre, 1970.
19. Elías. L.G., and Bressani, R. 1974. Nutritional factors affecting the consumption of leguminous seeds. Arch. Latinoam. Nutr. (Venezuela), 24, 365–378.
20. Bressani, R., and Elías, L.G. 1972. La calidad proteínica del maíz Opaco-2 como ingrediente de dietas rurales de Guatemala. Arch. Latinoam. Nutr. (Venezuela), 22, 577–594.
21. Bressani, R., and Elías, L.G. 1973. Development of new highly nutritious food products. In Rechcigl, M., Jr, ed., Man, food and nutrition. Cleveland, U.S., Chemical Rubber Company Press, 252–274.

The Problem of Legume Protein Digestibility

R. Bressani and L.G. Elías

Legume grains in general comprise an important part of the diet of populations living in tropical and subtropical areas. Their nutritional importance is even greater, as these populations have limited availability and consumption of foods of animal origin. In Latin America, most legume grains, but mainly *Phaseolus vulgaris*, are readily accepted and consumed almost daily by low-income groups. The main dietary component is a cereal grain, although in some areas it is replaced by starchy roots. In Central America bean consumption provides from 20 to 30% of total protein intake.

Experimental evidence has indicated conclusively that legume grains are the natural protein complement to cereal grains, and when both are ingested in the appropriate ratio, the protein quality is higher than that of the individual components. For beans and corn the best ratio by weight is 3:7 (1). However, intake in such a ratio is not common, with a tendency to be significantly lower in both quantity and frequency. The reasons are not known, although availability has often been suggested as the cause. Since the importance of legume grains as sources of protein is recognized, efforts are being made throughout the world to increase their productivity in the hope that this will increase availability and finally consumption. However, other causes may be responsible for the low intakes. It has been assumed that one such cause is the relatively low digestibility of the protein of legume grains.

This paper will, therefore, attempt to analyze the low protein digestibility of legume grains and will discuss the possible reasons for it. If these are identified and could be eliminated, beans will make a better nutritional contribution than that made during the last 4000 years since their consumption apparently was initiated.

The Problem of Legume-protein Digestibility

Most nutritional and biochemical studies carried out with legume grains have dealt mainly with two factors that are important in determining their protein quality. One consists of the antiphysiological substances present in legume grains, of which the trypsin inhibitors, amylase inhibitor, and hemagglutinins are the most important. The second is the well-documented deficiency of sulfur-containing amino acids in legume grain protein. Of the two factors, the antiphysiological compounds could be responsible for low protein digestibility. However, these substances are destroyed completely or to a large extent by heat treatment; therefore, it can be assumed that in cooked foods they are not responsible for such a characteristic. On the other hand, because legume grains are heat-treated

Table 1. Apparent protein digestibility of various legume grain species fed to rats (2).

	Protein digestibility, %
Glycine max	81.1–83.0
Lens esculenta	80.2
Vigna sinensis	76.5–81.4
Cajanus cajan	76.5–76.6
Cicer arietinum	76.3
Dolichos lablab	74.5
Pisum sativum	70.7–76.0
Phaseolus vulgaris	68.7–74.2
Cajanus indicus	47.7–75.3

Table 2. Fecal nitrogen and apparent protein digestibility in human adults fed egg protein and split peas with and without added methionine (3).

Protein source	Nitrogen			Apparent protein digestibility, %
	Intake, g	Fecal, g	Absorbed, g	
Egg	5.60	0.81	4.79	85.6
Split pea	5.47	1.16	4.31	78.8
Split pea + Met	5.95	1.19	4.76	80.0

Table 3. Fecal nitrogen of children and young dogs fed various protein foods with cooked beans (4).

Diet	Nitrogen, mg/kg per day		Fecal N of N intake, %
	Intake	Fecal	
Children			
Milk	387	70	18.1
25% Milk + 75% (maize + beans)	358	98	27.4
10% Milk + 90% (maize + beans)	353	134	38.0
100% (maize + beans)	347	107	30.8
Dogs			
Maize	520	169	36.3
Maize + black beans	635	254	40.0

Table 4. Fecal nitrogen losses from milk and *P. vulgaris* fed to children (5).

Protein source	Nitrogen balance, mg/kg per day		
	Intake	Fecal	Absorbed
Milk	236	46	190
Cooked black beans	227	81	146

before consumption and also for evaluation of their protein quality, and extended heat-treatment decreases protein digestibility, the real cause could be the thermal process used.

The protein digestibility of various legume grain species is shown in Table 1, as determined in the laboratory rat (2). These results show that there is a great variation within the same species. For example, in *Cajanus indicus* the values range from 4.7 to 75.3%. Large differences in protein digestibility are also evident between species. All values were obtained from feeding cooked samples. Therefore, it cannot be assumed the variation is due to residual levels of anti-nutritional factors, unless the heat treatment employed was less effective in some species than in others.

The results of the studies on human adults fed Alaska split pea (*Pisum sativum*) (3) are shown in Table 2. Feeding split pea at a level of nitrogen intake similar to that provided by egg, increased fecal nitrogen excretion from 0.81 to 1.16 g/day. Apparent protein digestibility for egg was 85.6% and that for split pea was 78.8%. For egg, fecal nitrogen was 14.5% of intake, but for split pea, fecal nitrogen was 21.2% of intake. The results also show that added methionine did not change fecal nitrogen excretion, which in this case represents 20% of intake. Results of various studies with children fed *P. vulgaris* in com-

bination with other foods are shown in Table 3. Fecal nitrogen increased as milk nitrogen intake decreased and on a relative basis fecal nitrogen varied from 18.1 to 38.0% of nitrogen intake. Not all the effect can be attributed to bean protein, because it was given in combination with maize. However, nitrogen loss increased, and beans may be responsible to some degree (4).

Results in the lower section of the table were obtained with young dogs. In the case of corn, fecal nitrogen amounted to 36.3% of the intake. In the second experiment, in which 32% of the nitrogen intake was from beans, fecal nitrogen increased to 40% of the nitrogen intake (4), these figures representing significant losses of nitrogen.

Additional information for children is summarized in Table 4 (5). Fecal nitrogen for milk was equivalent to 19% of milk nitrogen intake, but for beans, it was equivalent to 36% of nitrogen intake. The losses of nitrogen in feces resulted in a nitrogen retention for milk of 80.5% of intake and only 64.3% for bean.

These results, from both animals and humans, suggest that legume grain protein is in general of low digestibility. However, it is of interest to analyze the results for apparent protein digestibility of other vegetable protein sources. Table 5 summarizes some results obtained in children fed vegetable proteins (6). Apparent protein digestibility values of these proteins ranged from 68 to 85%, which is also the range of protein digestibility values found for the legume grains. Although some of the diets contained legume grains, others did not, or were single foods. In view of this, one may ask if the low digestibility of legume grain protein is a particular characteristic of legume grains, or if it represents a characteristic common to all or almost all vegetable protein sources.

The Role of Antiphysiological Factors

In the previous section, it was suggested that antiphysiological factors, such as trypsin and amylase inhibitors, could be partially responsible for the low digestibility of the protein in legume foods. Even though these factors are destroyed by heat, it is of interest that when legume grains are fed raw, they affect protein digestibility. Table 6 summarizes the results of various investigators (7–12, 23). It is evident that raw *Phaseolus* are poorly digested with values ranging from as low as 15.6–56%. However, genera such as *Vigna* do not show low digestibility values when fed raw. *Vigna sinensis* has significantly lower levels of trypsin inhibitors than *Phaseolus* species. Although it has been indicated that no correlation seems to exist between trypsin inhibitory activity and protein digestibility (7), it is clear that the presence of such activity will interfere with the process of protein breakdown in the gastrointestinal tract. The lack of correlation may be explained by recent findings that suggest two types of trypsin inhibitory activity. One is heat-labile or true trypsin inhibitor activity, and the second is heat-resistant, probably due to tannins or polyphenols (13). However, independent of these, there are other factors and conditions that contribute to a variable degree to changes in protein digestibility.

The Role of Heat Treatment on Protein Digestibility

The variation in protein digestibility shown for legume grains is probably the result of various factors, which can be inherent in the seed; or a result of handling and storage of the seed; or result from the thermal process utilized to prepare the seed for biological evaluation.

As indicated before, the extent and length of the thermal process may damage the protein in terms of its quality, of which protein digestibility is a part. The results (Table 7) show the importance of cooking (11). The low value for the raw seed is expected because it still contains active antiphysiological compounds. In the cooked samples, the digestibility varied as the pressure used in cooking. A pressure of 15 lb/inch^2

Table 5. Apparent and true protein digestibility of various vegetable protein sources (6).

Protein source	Digestibility	
	Apparent, %	True, %
Milk, wheat flour, chick pea flour, and lentil flour	70	86
Milk, wheat flour chick pea flour, and soy flour	71	88
Milk, wheat flour, chick pea flour, and split pea flour	68	85
Corn and cottonseed flour	68	84
Corn and soybean flour	74	91
Corn, soybean, and cottonseed flour	74	92
Corn	69–75	—
Wheat	85	—
Rice	79	—
Milk	82	92
Egg	79	98

Table 6. Protein digestibility of raw and cooked legume foods.

Legume food	Scientific name (genus and species)	Protein digestibility: Raw, %	Protein digestibility: Cooked, %	Type	Reference
Common bean (red)	*P. vulgaris*	49	71	in vitro	(10)
Common beans (red)	*P. vulgaris*	56	83	in vitro	(10)
Common beans (black)	*P. vulgaris*	55	80	in vitro	(10)
Common beans (white)	*P. vulgaris*	52	91	in vitro	(10)
Common beans	*P. vulgaris*	43.5	80.9	in vivo	(8)
Common beans (black)	*P. vulgaris*	15.6	71.2	in vivo	(11)
Common beans (white)	*P. vulgaris*	42.7	74.9	in vitro	(23)
Common beans (black)	*P. vulgaris*	41.1	68.1	in vitro	(23)
Common beans (red)	*P. vulgaris*	36.3	72.3	in vitro	(23)
Soybeans	*Glycine max*	70.1	85.4	in vivo	(7)
Soybeans	*Glycine max*	82.9	89.7	in vivo	(9)
Cowpea	*V. sinensis*	79.0	82.6	in vivo	(7)
Cowpea	*V. sinensis*	73.2	72.4	in vivo	(12)
Lima beans	*P. lunatus*	34.0	51.3	in vivo	(7)
Pigeon peas	*C. cajan*	59.1	59.9	in vivo	(7)

Table 7. Effect of various types of cooking process on the digestibility of *P. vulgaris* (black) (11).

Treatment	Digestibility
Raw	15.6
Cooked in distilled water, 85 °C, 2 h	48.7
Cooked in 0.1% CH_3COOH, 85 °C, 2 h	46.6
Cooked in 0.1% $NaHCO_3$, 85 °C, 2 h	52.9
Autoclave 15 psi, 30 min	71.2
Autoclave 15 psi, 30 min, no seed coat	73.0

Table 8. Effect of thermal treatment on the protein digestibility of *V. sinensis* (cowpea) (12).

Thermal treatment	% digestibility	Available lysine, g/16 g N
None	73.2	6.4
Autoclaved 16 psi, 121 °C, 15′	72.4	5.7
Toasted 2 30′, 210 °C	76.1	4.8
Toasted 3 20′, 230 °C	73.4	4.9
Toasted 1 30′, 240 °C	65.0	2.9
Extrusion	80.2	5.6

(1.0545/cm²) is much more effective than atmospheric pressure, or longer cooking time. The reason for these differences may be the degree to which the antiphysiological factors were inactivated by heat.

To eliminate this variable as responsible, similar studies were made with other thermal treatments applied to *V. sinensis* (12). This legume grain contains only small amounts of trypsin inhibitors. The results (Table 8) show that the raw seed has a protein digestibility essentially the same as the sample that was autoclaved. Excessive toasting, in terms of higher temperature and time, decreased digestibility from 76 to 65%, whereas extrusion cooking gave a product the highest digestibility of 80%. Heat obviously decreased the quality of the protein, damaging to a large extent the available lysine content as shown. However, a process such as extrusion cooking, involving a high-temperature short-time process, improved the digestibility of the protein in a way yet unknown. It is possible that this treatment is capable of destroying cell walls, thus making the contents more available to enzymatic action, or the carbohydrate fraction was made more susceptible to hydrolysis.

Cooking time under pressure is also a factor that may influence protein digestibility, because optimum conditions vary according to the legume grain species (14). For example, Table 9 shows that for *P. vulgaris* a cooking time of 45 min under pressure increased protein digestibility slightly (73–74.2%), whereas the same conditions decreased the protein digestibility of *V. sinensis*

Table 9. Effect of pressure cooking time on protein quality and digestibility of three legume grain species (14).

	Pressure cooking time, min			
	15		45	
Legume grain species	PER[a]	% PD[b]	PER	% PD
P. vulgaris (black)	0.70	73.0	0.94	74.2
V. sinensis (cowpea)	1.37	77.5	0.84	74.3
C. cajan (pigeon pea)	1.42	80.4	1.61	78.1

[a]PER, protein efficiency ratio.
[b]PD, protein digestibility.

from 77.5 to 74.3% and of *Cajanus cajan* from 80.4 to 78.1%. The changes are in general small; however, they are consistent.

The application of heat may be interpreted as having a double effect, taking place one as a consequence of the other. The first effect is to decrease and eliminate the activity of the anti-physiological factors, as is well-documented. The second is to increase amino acid availability from the protein. This is shown in Table 10 for *P. vulgaris* (8). The greatest increase in availability was observed for the sulfur-containing amino acids, methionine and cystine, amino acids that are present in high concentration in the trypsin inhibitors. It should be recalled, however, that excessive heat may decrease amino acid availability. As was shown, the effects of heat can be measured by the extent of inhibitor inactivation, by the increase in sulfur amino acid availability, and by a decrease in lysine availability.

Finally, storage time also influenced protein digestibility of legume grains. Some results are shown in Fig. 1. In these studies *P. vulgaris* was stored for 0, 3, and 6 mo. After the specified storage time, they were soaked in water and cooked under pressure for 10, 20, and 30 min. The results show that for all storage periods, longer cooking time decreased protein digestibility. Likewise, storage time also affected protein digestibility at each cooking time (15).

These results, therefore, show that there are too many factors influencing the protein digestibility of legume grains, making it absolutely necessary to study them individually and under well-controlled and known processing conditions. The lack of standard methods of processing, and of information defining or describing the conditions, may have contributed to the variation which has been reported in protein quality and digestibility. The progress of breeding programs for improved quality may be retarded unless steps are taken to standardize these conditions (16).

The Role of Water Soluble Nitrogen Fractions of Cooked Beans

To get more direct evidence on the digestibility of protein in legume grains, metabolic studies were carried out with dogs (17). They were fed six species of legume grains as part of the diet, each species being the sole source of protein providing 1.8, 2.9, or 3.7 g of protein per kilogram per day. Calorie intake was adjusted to 120 kcal/kg per day. Each level was fed for 8 days to get two nitrogen balance periods of 7 days each. The reference protein used was casein. After the 8-day trial, endogenous nitrogen excretions were determined by feeding a nitrogen-free diet. From the analysis of feces, urine, and food intake, apparent and true protein digestibility were calculated. The apparent protein digestibility for each species is shown in Fig. 2. The curves in the upper section of the figure are *P. vulgaris* of various seed coat colours. The lower graph represents three other species. The results show in both cases a significant difference in digestibility between the legume grains and casein. There are also differences between species, which persist independently of protein intake. In all species, protein digestibility increased with increased protein intake. In the upper group, red beans, and in the lower group pigeon peas, showed unexpected results in comparison with the other samples.

Table 10. Amino acid availability and protein digestibility in *P. vulgaris* (8).

Amino acid	Raw	Heated
Methionine	21.8	68.7
Cystine	36.6	80.6
Lysine	58.5	85.0
Leucine	47.6	85.7
Valine	46.0	84.8
Protein digestibility, %	43.5	80.9

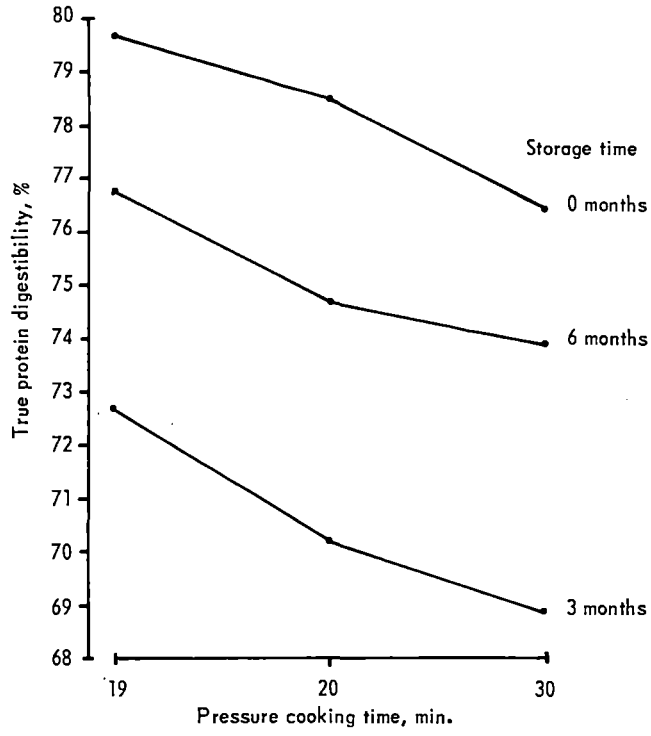


Fig. 1. Effect of storage time and cooking time on the protein digestibility of *P. vulgaris* (black).

Table 11. Fractionation of the legume grain nitrogen and of fecal nitrogen to water soluble and water insoluble nitrogen (17).

	In precooked legume grain		In feces	
Protein source	Water soluble N, %	Water insoluble N, %	Water soluble N, %	Water insoluble N, %
Black bean	15.2	84.8	35.3	64.7
Red bean	17.1	82.8	33.2	66.8
White bean	23.3	76.7	45.8	55.0
Casein	3.8	96.2	52.9	47.1
Soybean	57.4	42.6	43.7	56.2
Cowpea	40.9	59.1	49.1	50.9
Gandul	18.2	81.8	35.7	64.3
Casein	3.8	96.2	59.7	40.3

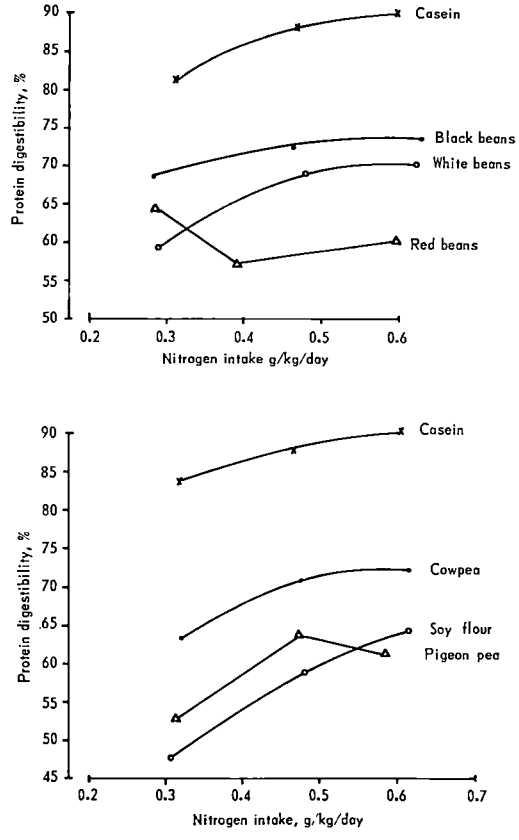


Fig. 2. Effect of protein intake on protein digestibility of some legume grain species.

During the last balance period, the feces were fractionated into water-soluble and insoluble nitrogen. The same type of nitrogen fractionation was done on the cooked legume grain flours. The results are presented in Table 11. Water-soluble nitrogen in the cooked flours ranged from 15.2% in the black bean to 57.4% in soybeans. The highest values occurred in soybean and cowpea. Insoluble nitrogen ranged from 42.6% in soybean to 84.8% in black beans. In feces, water-soluble nitrogen varied from 33.2 to 49.1% in the legume grains, and the insoluble nitrogen varied from 50.9 to 66.8%.

Using these figures, or the absolute amounts in the ingested and fecal nitrogen, the digestibility of the soluble and insoluble nitrogen fractions was calculated. These results are shown in Table 12. The values indicate that, with the exception of soybeans and cowpeas, digestibility of water-soluble nitrogen fraction was significantly lower than that of the insoluble nitrogen fraction. These in turn were higher than digestibility based on total nitrogen. These results were interpreted to mean that the protein that is more resistant to digestion is found in the water-soluble nitrogen fraction of precooked bean flour. It influences the digestibility of the fraction itself and of the

Table 12. Digestibility of the water soluble and water insoluble nitrogen of various legume grains (17).

	Apparent digestibility, %		
Protein source	Soluble N	Insoluble N	Total
Black bean	36.4	79.9	73.4
Red bean	14.2	64.3	60.1
White bean	41.2	78.7	70.3
Casein	—	94.5	89.1
Soybean	72.7	52.6	64.2
Cowpea	66.7	69.5	61.1
Pigeon pea	22.5	76.0	72.0
Casein	—	96.0	90.0

Table 13. Protein quality of various species of legume grains evaluated with and without their cooking broth (18).

Legume grain[a]	Avg wt gain[b], g		PER[c]	
	Cooking broth (with)	Cooking broth (without)	Cooking broth (with)	Cooking broth (without)
P. vulgaris (black)	6	29	0.20	0.88
P. vulgaris (red)	8	10	0.31	0.48
V. sinensis (snap pea)	32	52	1.04	1.24
C. cajan (pigeon pea)	36	51	0.98	1.46
G. max	94	113	1.69	1.80

[a]Cooking conditions: Soaking, 18 h, 15 psi, 20 min, and 121 °C; water–beans, 3:1 ratio.
[b]Twenty-eight days. Elías and Bressani (18).
[c]PER, protein efficiency ratio.

total nitrogen in the legume grain.

This interpretation, however, must be considered with some reservations. One limitation to this approach is that both the water-soluble and insoluble nitrogen fractions in the ingested bean may have undergone changes in solubility characteristics and are not identical in feces.

Therefore, a better identification is required in future work. However, the digestibility of the soluble nitrogen fraction is highly correlated with dry matter digestibility and nitrogen balance. The results of other studies seem to support the interpretation indicated above. In our laboratories for example, Molina and co-workers (15) found a negative relation between protein digestibility and a nitrogen fraction soluble in sodium chloride solution of cooked bean flours. Although the solvent used is different to that used for the dog study, the solubility values are similar. More recently Elías and co-workers (18) found (Table 13) that the protein quality of legume grains was higher when the cooking water is removed than when it is left with the grain. All samples were processed as shown at the bottom of the table. The extent of improvement in both weight gain and protein quality is in general greater with *P. vulgaris* than with cowpeas, soybeans, or pigeon peas. In view of these observations, in other studies the protein in the cooking broth was assayed for its protein digestibility, alone or together with the cooked grain. The results (Table 14) show that the lowest digestibility was that of the protein present in the broth. Although the differences between the values of beans with and without the cooking broth are small, it should be pointed out that it is difficult if not impossible to remove all cooking broth from cooked beans.

The Role of Proteins Resistant to Enzymatic Hydrolysis

Some studies have suggested that legume foods contain protein fractions that are resistant to proteolysis. For example, Jaffé and Hanning (19) found several purified bean protein fractions resistant to the hydrolytic activity of pepsin and papain. This observation could not be explained by the presence of trypsin inhibitor. In more recent studies, Seidl et al. (20) isolated a kidney bean globulin fraction that was resistant to hydrolysis by pepsin, trypsin, chymotrypsin, papain, ficin, hurain, and subtilisin. To isolate this fraction, a salt extract from beans was dialyzed against acetate buffer at pH 4.5. After denaturation of the globulin by heat or urea, only slight hydrolysis by the enzymes could be detected. Furthermore, the activity of all the enzymes on their respective substrates was inhibited by the bean globulin. This fraction amounted to about 30% of the extractable protein.

Referring to the studies on dogs in the previous section (17), the nitrogen fraction in feces named water-soluble is really a fraction soluble in salt solution because of the relatively high salt content of feces. This fraction occurred in feces in

Table 14. Protein digestibility of *P. vulgaris* evaluated with and without the cooking broth (12).

Sample	Protein digestibility, %
Cooking broth (alone)	65
Beans with cooking broth	72
Beans without cooking broth	75

Table 15. Protein quality and digestibility of black beans and its mutant white beans (*P. vulgaris*).

Legume grain[a]	Weight gain g/28 days	PER	Apparent digestibility
Black beans S-187 N (*P. vulgaris*)	33	1.16	75.0
White beans NEP-White 2 (*P. vulgaris*)	67	1.72	83.4

[a]Cooked in the autocalve at 121 °C (16 lb).

Table 16. Effect of seed coat colour on protein digestibility.

	Protein digestibility, %	
	Dogs[a]	Rats[b]
P. vulgaris (black)	72.5	68.9
P. vulgaris (red)	57.1	67.1
P. vulgaris (white)	68.9	71.2

[a]Bressani and Elias (second background paper in this book).
[b]Jaffe and Flores (11).

amounts ranging from 33.2 to 49.8%, and had digestibilities ranging from 14.2 to 72.7%, depending on the legume food studied. The lowest value was from red beans (*P. vulgaris*) and the highest from soybeans. It is possible that this nitrogen is the globulin fraction inhibiting proteolysis, and therefore, reducing protein digestibility.

Role of Seed-coat Pigments

Recent studies with bird-resistant sorghum have indicated that the pigments providing resistance also reduce the protein quality of the grains (21). Free phenolic compounds are common components in materials derived from vegetable products (22). Legume grains are no exception, and those commonly consumed are characterized by differences in the colour of the seed coat. Some evidence with respect to the colour of the seed has been obtained (23). Table 15 shows the protein digestibility of a white mutant (NEP 2), obtained by Co-60 irradiation of a black-coated bean, San Fernando variety. The digestibility was higher for the white-coated mutant than for the black-coated bean. Extensive chemical analysis including trypsin inhibitor activity, hemagglutinin concentration and amino acid analysis showed both to be essentially alike. This suggests that the pigments of the seed coat might be responsible. If the seed coat contained phenols or tannins, these could possibly react with the protein, decreasing its digestibility. Results from other studies are shown in Table 16. The values indicate that black beans have a higher protein digestibility and red beans the lowest of the group tested, both in dogs and rats. This effect was also observed in the digestibility of the dry matter ingested, with red-coated beans giving lower values than white- and black-coated beans (11, 17). The cause-effect relation is, however, not as simple, as other factors apparently play a role, such as length and conditions of storage, altering results.

Additional evidence is shown in Table 17 by data from our laboratories (24), which show that beans contain up to 17% lignified protein. If this is not digested, obviously protein digestibility will decrease. As indicated before, storage conditions influence the amount of protein that becomes lignified both in the seed coat and in the cotyledons, which will affect protein digestibility. Tannin content in faba beans has been reported to vary from 0.34 to 0.50% expressed as gallic acid equivalent, and from 0.34 to 0.46% as tannic acid equivalent (25). Values for common beans are not yet available.

Finally, in this respect it is of interest to indicate that an alcoholic extract of black beans inhibited growth performance in rats fed a casein diet to which the extract had been added (26).

The examples given, although small in number, probably have some significance in the context of the problem of protein digestibility of legume grains, and more studies should be carried out to corroborate the information presented.

Furthermore, these data demonstrate that knowledge of the causes of the low digestibility of

Table 17. Content of "lignified protein" in *P. vulgaris* (black-coated) (24).

Condition	Lignified protein (%) of total protein	
	Cotyledon	Seed coat
Stored at 4 °C (9 mo)	9.2	29.5
Stored at 25 °C (9 mo)	17.2	44.2

Table 18. Rate of passage of food residues from soybean protein and casein through the GI[a] tract of pigs at 4 or 10 weeks of age (28).

Diet	Age, weeks	
	4	10
Soybean protein		
Mean rate of passage, hours	19	45
Range	14–24	40–50
Casein		
Mean rate of passage, hours	42	45
Range	36–48	40–50

[a]GI, gastrointestinal.

legume grain protein is not readily obtained, and that the problem is more complex than it appears to be. New approaches to the problem must be developed.

The Role of the Rate of Passage of Food Residues

The rate of passage of food residue through the alimentary tract has been shown to be related to the type of diet fed (27). It is well accepted that the actual acid buffering capacity of a food is dependent chiefly on its protein content. Studies in gastric secretion have shown that soybean protein is capable of maintaining gastric pH above 3.0 for about 60 min longer than milk, fish, egg, or animal meat. It has been suggested that rate of passage is related to the difference in gastric pH response to diet. Therefore, if there is a buffering action of the protein, the activation of proteolytic enzymes would be delayed, reducing the digestibility of the protein. Two popular observations seem to provide some suggestions that bean intake produces acidity in many individuals, and that there is an increase in frequency and amounts of evacuation. Both conditions will decrease protein digestibility. The results of studies by Maner et al. (28) shown in Table 18 indicate that soybean protein had a mean rate of passage in young rats of 19 h compared to 42 h when casein was fed. This difference was age-dependent, as it was not found in older rats. In children, Rosales Arzu (5) found (Table 18) an increase in the number of evacuations as well as in their weight when they came from beans as compared to milk. This increase in both number and weight also involved greater loss of fecal nitrogen, resulting in lower protein digestibility coefficients.

These observations suggest, therefore, that the proteins in legume grains may have a lower digestibility through the action that all fractions, or a specific one, may have on gastric pH and on rate of passage through the gastrointestinal tract.

Table 19. Average number of weight of evacuations of children fed milk and beans (*P. vulgaris*) (5).

Diet	No. evacuations	Weight of evacuations, g
Milk	8	70
Beans	14	148

However, among habitually bean-consuming populations, the undesirable effects of bean consumption, such as acidity, flatulence, and the number and amount of evacuations, seem to be reduced, suggesting some type of adaptation to bean intake. The lesser effects may also be due to the way in which beans are prepared for consumption.

Conclusions

From the results presented it may be concluded that in legume grains there are at least four conditions that control digestibility of the protein. These are shown in Fig. 3. The evidence indicates that some species of legume foods when raw have a low protein digestibility. However, this is not a general characteristic for all species. This low digestibility is caused mainly by the trypsin inhibitors and hemagglutinin compounds. The result of the destruction of their inhibitory activity by heat is an increase in protein digestibility. The extent of improvement depends on the method used to prepare them for cooking, on the control of heat in terms of temperature, pressure, and time. As before, conditions vary with the age and the species of legume grain. Heat destroys the action of the inhibitors, and as they contain essential amino acids, particularly cystine, the effect is also to increase amino acid availability.

An additional increase in protein digestibility probably results from destruction of the tertiary structure of certain proteins, which offer resistance to enzymatic hydrolysis. The presence of this type of protein is probably a common feature of most vegetable proteins. Likewise, increased digestibility may result from cellular wall breakdown.

Finally, further increase in protein digestibility can be obtained by minimizing, controlling, or destroying the effects of protein-complexing substances in legume grains. Although it is recognized that more evidence should be obtained on all aspects, particularly the last one, the

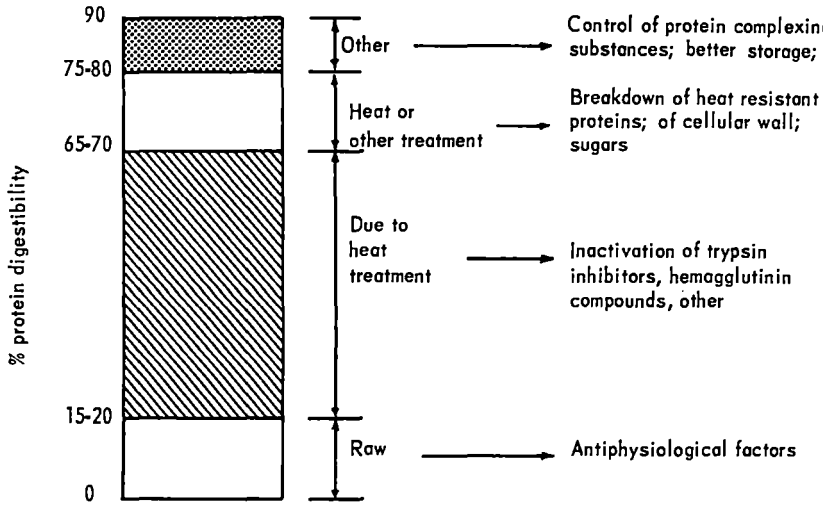


Fig. 3. Possible factors influencing the protein digestibility of legume.

evidence shows that poor storage conditions, colour of the seed coat suggesting the presence of phenolic compounds, phytic acid, sugars, saponins, and possibly other compounds, may inhibit protein digestibility and therefore, amino acid availability, reducing the efficiency of protein utilization.

Table 20 shows the practical significance of improved protein digestibility in legume grains and in vegetable proteins. The calculation shows the decrease in amount of wasted protein or wasted agricultural land that results from improved protein digestibility. Assuming an average yield of beans of about 1000 kg/ha with a protein content of 23%, the land provides 230 kg of protein. Using a protein digestibility value of 64% as obtained from human studies, and an improved protein digestibility value of 84% for the 230 kg of protein produced, 147 and 193 kg will be absorbed, respectively. This means that 83 and 37 kg of protein will be wasted in feces. The wasted protein is equivalent to 360 and 160 kg of beans, which indicates that from beans on 1 ha of land only 640 kg are of value for the low protein digestibility case, but 840 kg are of value when the digestibility increases. The picture is not complete, as it shows only the loss due to poor protein digestibility. However, if this is improved there are good possibilities to reduce protein losses in urine, through increased protein absorbed. This is shown at the bottom of the table. For this calculation, it was assumed that the amino acid pattern absorbed with digestibility 84% is the same as that with digestibility 64%. It is certain that urinary nitrogen will increase to some extent; however, for present purposes it was left the same. On this basis, nitrogen retention will increase from 37 to 82 mg/kg per day, indicating a more efficient use of the protein produced from 1 ha of land on beans. The urinary loss can also be reduced significantly by increasing the concentration of methionine in bean protein. However, improvement in the digestibility of the protein will also give a similar effect. Therefore, the understanding and solution of the problem of the low protein digestibility of legume foods, and of all vegetable proteins, is of great practical

Table 20. Efficiency of land utilization in terms of the protein from beans (*P. vulgaris*).

	Protein digestibility, 64%	Protein digestibility, 84%
Yield of beans/ha, kg	1000	1000
Yield of protein/ha, kg	230	230
Protein absorbed/ha, kg	147	193
Protein waste/ha, kg	83	83
Waste as beans/ha, kg	360	160
% land poorly utilized	36	16

NI[a]	FN[a]	UN[a]	NA[a]	NR[a]	NI	FN	UN	NA	NR
mg/kg per day					mg/kg per day				
227	81	109	146	37	227	36	109	191	82

[a]NI, nitrogen improved; FN, fecal nitrogen; UN, urinary nitrogen; NA, nitrogen absorbed; NR, nitrogen retention.

importance in terms of increasing the efficiency of land utilization for food production, particularly in view of the need to feed an increasing world population and of the position vegetable proteins are already occupying as food in the diet of the human population.

References

1. Bressani, R., Valiente, A.T., and Tejada, C. 1962. All-vegetable protein mixtures for human feeding. VI. The growth promoting value of combinations of lime-treated corn and cooked black beans. J. Food Sci. (U.S.), 27, 294.
2. Jaffé, W.G. 1950. El valor biológico comparativo de algunas leguminosas de importancia en la alimentación venezolana. Arch. Venez. Nutr. (Venezuela), 1, 107.
3. Esselbaugh, N.C., Murray, H.C., Hardi, L.W., and Hard, M.N. 1952. The replacement value of the Alaska field pea (Pisum sativum) for human subjects. J. Nutr. (U.S.), 46, 109.
4. Bressani, R. 1973. Legumes in human diets and how they might be improved. In Milner, M., ed., Nutritional improvement of food legumes by breeding: proceedings of the symposium sponsored by PAG, held at FAO, Rome, Italy, 3–5 July, 1972. New York, U.S., Protein Advisory Group, United Nations, 15–42.
5. Rosales Arzú, A.M. 1972. Estudio sôbre la calidad proteínica del frijol y la de tres fracciones derivadas por solubilidad diferencial en niños pre-escolares. In INCAP. Thesis, Escuela de Nutrición, Guatemala.
6. Viteri, F., and Bressani, R. 1972. The quality of new sources of protein and their suitability for weanling and young children. Bull. W.H.O. (Switzerland), 46, 827.
7. Jaffé, W.G. 1950. Protein digestibility and trypsin inhibitor activity of legume seeds. Proc. Soc. Exp. Biol. Med. (U.S.), 75, 219.
8. Kakade, M.L., and Evans, R.J. 1966. Growth inhibition of rats fed raw Navy beans (*Phaseolus vulgaris*). J. Nutr. (U.S.), 90, 191.
9. de Muelenaere, M.J.H. 1964. Studies on the digestion of soybeans. J. Nutr. (U.S.), 82, 197.
10. Jaffé, W.G. 1973. Factors affecting the nutritional value of beans. In Milner, M., ed., Nutritional improvement of food legumes by breeding: proceedings of the symposium on PAG, United Nations, New York, U.S., 3–5 July 1972. New York, U.S., Protein Advisory Group, United Nations, 43–48.
11. Jaffé, W.G., and Flores, M.E. 1975. Cocción de frijoles (Phaseolus vulgaris). Arch. Latinoam. Nutr. (Venezuela), 25, 79.
12. Elías, L.G., and Bressani, R. 1977. The effect of various types of heat treatment on the protein quality of cowpea (V. sinensis). (To be submitted for publication.)
13. Elias, L.G., de Fernández, D.G., and Bressani, R. 1977. Studies of beans on the nutritive value of its protein. J. Food Sci. (U.S.). (Submitted for publication.)
14. Elías, L.G., Hernandez, M., and Bressani, R. 1977. The nutritive value of pre-cooked legume flours processed by different methods. (To be submitted for publication.)
15. Molina, M.R., de la Fuente, G., and Bressani, R. 1975. Interrelationships between storage, soaking time, cooking time, nutritive value and other characteristics of the black bean (P. vulgaris). J. Food Sci. (U.S.), 40, 587.
16. Bressani, R. 1975. A new assessment of needed research. In Milner, M., ed., Nutritional improvement of food legumes by breeding. New York, U.S., John Wiley & Sons Inc., 381.
17. Bressani, R., Elías, L.G., and Molina, M.R. 1977. Estudios sôbre la digestibilidad de la proteína de varias especies de leguminosas. Arch. Latinoam. Nutr. (Submitted for publication.)
18. Elías, L.G., and Bressani, R. 1977. Composicion qüimica y valor nutritivo de algunas leguminosas de grano de El Salvador. (To be submitted for publication.)
19. Jaffé, W.G., and Hanning, K. 1965. Fractionation of protein from kidney beans (Phaseolus vulgaris). Arch. Biochem. Biophys. 109, 80.
20. Seidl, D., Jaffé, M., and Jaffé, W.G. 1969. Digestibility and proteinase inhibitory action of a kidney bean globulin. J. Agric. Food Chem. (U.S.), 17, 1318.
21. Armstrong, W.D., Featherston, W.R., and Rogler, J.C. 1973. Influence of methionine and other dietary additions on the performance of chicks fed bird resistant sorghum grain diets. Poult. Sci. (U.S.), 52, 1593.
22. McLeod, M.N. 1974. Plant tannins — their role in forage quality. Nutr. Abstr. Rev. (England), 44, 803.
23. Elías, L.G., Moh, C.C., and Bressani, R. 1973. Protein quality of a white coated mutant from irradiation of a black coated variety of beans (P. vulgaris). INCAP, Annual Report 1973.
24. Molina, M.R., Batten, M.A., and Bressani, R. 1977. Heat-treatment; A process to control the development of hard-shell in black beans (Phaseolus vulgaris). (Submitted for publication.)
25. Kadirvel, R., and Cladinin, D.R. 1974. The effect of faba beans (Vicia faba) on the performance of turkey poults and broiler chicks from 0 to 4 weeks of age. Poult. Sci. (U.S.), 53, 1810.
26. Núñez, E.I. 1975. Efecto de varios solventes sôbre la extracción de las diferentes fracciones protéinicas del frijol y digestibilidad de las mismas. In CESNA/INCAP. Thesis postgraduate course, Guatemala.
27. Osmon, K.L., Belfour, D.C., and Wharton, G.K. 1957. The effect of common dietary protein on gastric secretion. Am. J. Gastroenterol. (U.S.), 28, 432.
28. Maner, J.H., Pond, W.G., Loosli, J.K., and Lowrey, R.S. 1952. Effect of isolated soybean protein and casein on the gastric pH and rate of passage of food residues in baby pigs. J. Anim. Sci. (U.S.), 21, 49.

A Short Summary of Methods of Testing Grains, Seeds, and Related Products for Protein Content

Philip C. Williams

The purpose of this short paper is to summarize and update the methods most commonly used for the estimation of protein in grains, seeds, and related products. As a single parameter, the total protein content of a food substance probably gives more information concerning the nutritional and processing quality of the material than any other physical or chemical test. It is a well-known fact that the "protein content" is arrived at by determining the total nitrogen content of the material, then multiplying this figure by a factor that is reputed to convert nitrogen to the equivalent of protein present. The nitrogen-to-protein conversion factor depends primarily on the true percentage of nitrogen in the pure proteins of the material. Since pure proteins are very difficult to isolate and characterize, the accuracy of the "protein content" figure remains largely subjective. However, the total nitrogen content is a value that can be determined with a high degree of accuracy, largely due to the painstaking research of Johann T. Kjeldahl, who devoted most of his latter years to the development of an accurate method for the determination of this all-important element (1).

The success of Kjeldahl's work is reflected in the fact that 93 years after its publication, the Kjeldahl test remains the absolute standard of accuracy in the determination of nitrogen, and all other nitrogen tests have to pass the rigorous examination of statistical comparison with the Kjeldahl test before serious consideration is given to their acceptance as a reliable method of analysis. It is probably the most widely used chemical test in the world, as applied to agronomic materials. Usually 0.5–5 g of sample is used, and the amount may be varied, depending on the amount of nitrogen present.

The Kjeldahl Test

The macroscale Kjeldahl test

The Kjeldahl test consists, in brief, of a conversion of all amino acids and amide nitrogen to ammonia by oxidative digestion in the presence of boiling concentrated sulfuric acid, which is diluted with water after cooling. The ammonia is liberated from the resulting solution of ammonium sulfate by neutralization with a strong alkali, and is quantitatively steam-distilled into a solution of dilute acid of known strength. The amount of acid equivalent to the ammonia is determined by back-titration using dilute alkali of known strength. The chief modifications to the original procedure since its invention have been (*a*) the introduction of a catalyst to hasten digestion (2); (*b*) Gunning's (3) suggestion of the addition of a salt (usually potassium or sodium sulfate) to increase the boiling point of the digestion mixture. The salt was originally intended as a replacement for mercury, but the digestion time has been found to be even shorter if both a salt and a catalyst are used in the digestion mixture; (*c*) Winkler proposed the replacement of standard acid with boric acid as a receiving solution, into which the ammonia is distilled (4). The latter modification, by eliminating the use of standard alkali, streamlined the process considerably, and is the method generally preferred in large-scale testing operations. A great deal of research has been expended on the various aspects of the Kjeldahl test, including the temperature and length of digestion, the influence of salt concentration, various catalysts, etc., most of which research has been documented and summarized in an excellent monograph by Bradstreet (5). The salient points of the test in its modern format are described in the Association of Official Analytical Chemists (AOAC) or American Association of Cereal Chemists (AACC) recommended test procedures (6, 7).

Micro-Kjeldahl tests

As the title implies, micro-Kjeldahl tests are small-scale Kjeldahl tests, using smaller amounts of sample, microdigestion, and microdistillation. From 50–250 mg are commonly used weights of starting material, and 100-ml flasks. For accurate and consistent results, the heat input at the digestion stage should be equivalent to that of macro-Kjeldahl digestion. This can be achieved by a short (15–20 min) heating period, preferably on an open element microdigester. The temperature of the boiling digest should be 365–380 °C.

An alternative method of microdigestion, which is increasing in popularity, involves a more lengthy period on a heating block, whereon the digestion is carried out in combustion tubes. The use of multidigestion blocks is increasing, but the operator should carefully evaluate each new unit to assess the degree of uniformity of digestion across the block.

The degree of digestion can be best assessed by

the analysis of pure compounds, such as ammonium sulfate or urea, both of which are available at a high degree of purity. Overdigestion occurs as frequently as underdigestion, and nitrogen can be lost in varying amounts by heating at over 400 °C. The catalysts used for microdigestion are basically the same as those used in the standard macro-Kjeldahl process. The most common practice is to employ digestion tablets, which contain potassium sulfate, together with the catalytic ingredients. Generally mercuric compounds are the most consistent catalysts, although the more mettlesome selenium is fairly widely used in the form of standardized tablets or "Keltabs." The amounts of mercury or selenium used in microdigestion are considerably less than in macrosystems, where the extreme toxicity of both chemicals presents an expensive problem in pollution control. Microdigestion is often aided by the use of hydrogen peroxide in addition to the concentrated sulfuric acid. Continuous digestion equipment is marketed by the Technicon Corporation. This equipment utilizes the usual reagents, but reports as to the efficiency and reproducibility of the digestion indicate variable acceptance of this method.

Microdistillation is usually accomplished by means of a steam generator and specialized glass microstills, into which are incorporated traps to avoid the loss of ammonia during the addition of the alkali. The Parnas–Wagner and the Markham stills have received the widest acceptance, with the latter "getting the nod" for simplicity. Microdigests are washed into the stills, which are stoppered, the alkali added, and the ammonia distilled into (usually) boric acid solution. The microstills can be washed very effectively by dipping the ends into distilled water and allowing the cooling still to siphon the pure water through the condenser and splash head, at least twice. The basic details of the micro-Kjeldahl test are covered in references 6 and 7.

Kjeldahl "compacts"

Recently two companies have introduced systems for macro-Kjeldahl determination that are basically designed to provide the small-space laboratory with the wherewithal to accomplish macro-Kjeldahl testing without the expensive fume extraction and plumbing necessary to the traditional Kjeldahl testing facility. The Foss Company of Denmark introduced their KelFoss system, which is a semiautomated Kjeldahl system, capable of performing a test every 12 min, after an original 15-min digestion time lag. This elegant equipment performs a continuous automatic titration against liberated ammonia and provides a digital printout of the results (8).

Finally, the Tecator-Udy Company of Boulder, Colo., U.S., presented a Kjeldahl package incorporating a block digester, which, in conjunction with an ingenious distillation apparatus, provides the equivalent of a full-scale Kjeldahl test at the rate of 8–12 tests per hour. Both of these systems achieve their rapid digestion by employing a high salt-concentrated sulfuric acid digestion solution, which also requires a high concentration of catalyst (about two to three times the normal level).

Alternative Methods of Testing for Protein Content

Although the Kjeldahl test is widely accepted as the standard method for the estimation of protein in grains and related products, and is likely to remain as such, it does suffer from several shortcomings, the most important of which is the lengthy time involved (1.5–2 h for a single test). The current worldwide concern for nutritional standards, coupled with an increasing demand for protein information in large-scale plant-breeding programs have stimulated research into the development of more rapid methods of testing for protein. During the last 20 years a number of chemical and physicochemical procedures have evolved that enable a competent operator to achieve a result in a few minutes at the expense of little of the stated accuracy of the Kjeldahl test.

Because all of the modern rapid tests are standardized and compared to the Kjeldahl test by the application of mathematical statistics, the operator should be aware that the accuracy of these procedures is vulnerable not only to the sources of error inherent in the specific tests, but also to the vagaries, both of the Kjeldahl process and of statistical principles. The chief sources of error of the Kjeldahl test and the popular dye-binding capacity test (to be described below) are summarized in a recent paper that also illustrates the impact of errors of certain magnitudes on the eventual results obtained (9).

Chemical methods

Classical wet chemistry is an art and a science that is in the process of becoming viewed with some disfavour, partly as a result of the nature of the chemicals themselves, partly as a result of the time involved in even so-called short processes, and partly because a certain amount of basic skill is required to achieve consistent and accurate figures. Nevertheless, there are two well-defined chemical systems for the estimation of protein that are significantly more economical in time, chemicals, and equipment than the true Kjeldahl process. These systems are classified as tests that involve true chemical reaction, and those that

depend upon the affinity of proteins for certain dyes. In both cases the final assay of the protein is colourimetric.

(1) *Tests that involve true chemical reaction* — For the purpose of this short discussion, "chemical reaction tests" are defined as tests in which one or more of the reagents undergoes a significant change in chemical structure. Included in this group are the automated sodium phenate and sodium dichloroisocyanurate procedures, the Nessler test, the salicylic acid procedure, and the Biuret test.

(2) *Tests that involve digestion* — The following tests consist of a series of reagents that have been applied to the estimation of ammonia produced during digestion. These include:

(i) The alkaline sodium phenate procedure — This reaction is applicable by either a manual technique (10) or an automated procedure (11, 12). In either case the colour development depends on the oxidation of phenol to indophenol, using hypochlorite in alkaline solution. The blue colour is read at 625 nm. Commercial bleach solutions are frequently used as a source of hypochlorite.

(ii) Trinitrobenzenesulfonic acid — This reagent has been described by Gehrke and co-workers (13) at an alternative to the sodium phenate method. The colour is compared at 398 nm, and is readily adaptable to automation.

(iii) Sodium dichloroisocyanurate — This colour reaction has been applied to the determination of ammonia in digests of plant materials (14). The reagents include sodium salicylate and sodium nitroprusside with colorimetry at 660 nm.

(iv) The Nessler reaction — The orange-coloured complex that is formed by the combination of ammonia with potassium mercuric iodide in alkaline solution is stable for several hours, and can be used to determine ammonia over a wide range of concentrations (15). Turbidities may be introduced by the presence of excessive concentrations of a number of metallic ions, including iron, nickel, cobalt, manganese, chromium, and aluminum, all of which form insoluble hydroxides. Turbidities may be induced to varying degrees by a slow rate of addition of the alkaline reagent to the test solution. As a result of this shortcoming the Nessler reagent has not achieved wide acceptance in automated nitrogen determination. Note that procedures involving digestion are measuring nitrogen in exactly the same manner as the standard Kjeldahl test, whereas other tests involving true chemical reaction depend upon a regression analysis for the computation of results.

(3) *Tests that depend on extraction* — Proteins are extracted from pulverized grain to varying degrees, depending partly on the mean particle size of the material and partly on the solvent. In the case of ground wheat, over 90% of the total protein can be extracted with dilute alkali. Other solvents that are reputed to be effective include dilute acetic acid and solutions of sodium salicylate. The extractability of protein by these solvents is more variable, and recoveries of from 60 to over 90% have been reported by extraction with salicylate.

(i) The Feinstein–Hart procedure (16) — This is a turbidimetric test that involves the extraction of protein from finely ground grain, using dilute sodium hydroxide followed by the addition of sulfosalicylic acid. The test has never achieved widespread acclaim, mainly due to the superior precision of the Biuret test.

(ii) The Biuret test — Cupric ions are bound by the peptide linkages present in proteins to form a purple-coloured complex. The colour is stable for several weeks, and has been adapted to the determination of protein in cereals and related products (17, 18). The extraction and reaction of the proteins is affected to a certain extent by the texture of the grain, and the mean particle size of the pulverized material, particularly in the case of wheat (19). More recently, the Biuret reaction has received renewed attention and the work of Johnson and co-workers (20), Noll and co-workers (21), and Simmonds and Ronalds (22) has resulted in the development of elegant automated equipment capable of achieving a complete test in about 5–6 min, including time for sample preparation. In tests of this type the degree of extraction and reaction do not influence the accuracy of the test, provided that a constant solvent–reaction time system is practiced. The most serious factors that influence the accuracy and precision of the Biuret test include the clarification of the reaction solution, since even slight turbidities can lead to erroneously high results, and the fiber content of the sample. Brown and green off-colours may be generated to varying degrees by fiber components. These tints are generally not experienced in the analysis of wheat and wheat products, but they may attain significance in the analysis of barley and oats of low test weight.

(iii) The Lowry test — This test is based on the Folin phenol reagent. It was adapted to the analysis of cereals and other plant products by Jennings (18) as a method for the analysis of more fibrous plant materials.

(4) *Dye-binding methods*

(a) The Udy method (23) — This test is based

on the removal of dye from solution, as a result of the binding of the dye chemical by the proteins present in suspensions of pulverized seeds and other plant material. The test occupies about 4.5–5 min. The accuracy and precision does not depend on extraction of proteins, and due to the rapid macerating action of the reaction cell the test is affected to only a minor degree by differences in the particle size of the material to be tested. After reaction the suspension is clarified, and a small amount of the clarified dye solution is compared spectrophotometrically to assess the loss of intensity in the colour of the dye solution. This is directly proportional to the protein content of the material. The Udy system is applicable to fairly large-scale testing. It utilizes the dye acid orange 12. This dye possesses a single sulfonic acid grouping, which reacts with the basic amino acids present in protein suspensions. As a result of this feature the method also serves as a system for the screening of large populations of early generation plant material for lysine content. The dye-binding capacity test (DBC) for proteins has received approval by the AACC for use as an official method of protein estimation.

(b) The Prometer (24) — This equipment is marketed by the Foss Electric Co., Hillerod, Denmark. It utilizes the same principle as the Udy method. The equipment incorporates a semiautomatic clarification process, and the entire test occupies 6–7 min. It has achieved reasonable success in Europe, but in North America it has been overshadowed by the faster, less complicated Udy process.

(c) The rapid protein meter — This equipment has been developed by the British Flour Milling and Baking Research Association, and utilizes the same principle as the Udy and Prometer methods. In this case the dye, di-acid light green is preferred (25).

Physicochemical methods

The advantages of the above chemical methods over the standard Kjeldahl process are associated directly with the saving of time, chemicals, and effort. The decrease in the total time per test is significant, and for most purposes is sufficient to allow on-the-spot analysis for protein. These tests should be referred to at best as "semirapid" since for large-scale continuous marketing of grain on a protein basis from road or rail vehicles, the above chemical tests, "rapid" though they may seem, would cause serious delays in the unloading of grain into elevators, both in the country and at the seaboard. Furthermore, the daily throughput of the faster chemical method is about 70–80. Physicochemical methods of analysis, although in their relative infancy, represent the way of the future for maximum throughput and speed per test, while retaining levels of accuracy and precision equal to the best of the above chemical methods.

(1) Neutron activation analysis — This technique was introduced by the Kaman Sciences Corporation about 7–8 years ago. The principle involves the activation of nitrogen by fast neutrons, followed by the assay of the radiation from the activated material as the isotope ^{13}N decays. The application has been described by several authors, including Johansson and coworkers (26) and Niemann and Neumuller (27). The equipment is automated, and is applicable to large-scale testing, rather than to the execution of individual rapid tests. Interferences experienced from phosphorus, silicon, and potassium are minimized by allowing up to 10 min for sample radiation decay before the actual assay of nitrogen starts. Accordingly, the time for a single test is about 15 min, but the automation allows for a daily throughput of up to 1000 samples per day. Sample size is about 20 g and grinding is not necessary. The use of large-scale automated systems such as this require a simple but comprehensive method of sample identification. Reproducibility of the test process is equivalent to that of the Kjeldahl test.

(2) Proton activation (29) — Probably the most sophisticated method of protein analysis ever devised depends on the nuclear reaction $^{14}N + p \rightarrow {}^{14}O + n$, which is induced by the bombarding of the nitrogen atoms with protons at a specific energy level. The energy level of the protons is such that no other nuclear reaction leading to ^{14}O is possible, so that the process receives no interference from extraneous sources. The ^{14}O decays to ^{14}N by the emission of a positron, and a 2.31 MeV gamma ray. The 2.31 MeV gamma ray is specific to ^{14}O, so that the intensity of 2.31 MeV rays permits an estimation to be made of the total nitrogen present in the sample. Although the initial expense of the equipment is at present fairly high, the process may become economical to operations processing samples at the rate of 1500–2000 per day.

Both of the above techniques are applicable only to large-scale operations. Both processes give a measure of the total nitrogen present, and can, therefore, be said to give the identical figure to the standard Kjeldahl process. The sources of error are generally fewer than in the Kjeldahl system, but accurate sampling remains a necessity. It should be borne in mind that large-scale operations that may be able to justify the use of this type of equipment are often required to quote

protein information on a constant moisture basis, or on a dry matter basis. In either case it becomes necessary to carry out an equal number of moisture tests, which may place a considerable strain on an operation that has not catered for the extra moisture testing.

(3) Infrared reflectance spectroscopy — Infrared reflectance spectroscopy (IRS) is a recent and dramatic introduction to the tools available for the analysis of grains for protein, moisture, and oil (29, 30). The testing process is very rapid (about 45–60 s, including sample preparation), uses no chemicals or irradiation, and has an accuracy and reproducibility equal to the best of the non-Kjeldahl methods. The principle of analysis is as follows: the energy of the peptide linkage is associated with the absorption of light at about 2.16 μm. Because the number of peptide linkages present in a protein is directly related to the amount of protein present, a measurement of the amount of absorbance at this wavelength can be utilized to measure the amount of protein present.

IRS instruments contain a light source, usually a tungsten filament. Light passes through special optical glass filters that allow the passage of light over a narrow wavelength band. Light passing through the filters is reflected from a standard reference plate, usually of Teflon or ceramic, and impinges on lead sulfide detector cells, situated at a set distance above the standard. The reflected light, converted into electrical energy, is amplified and stored in a series of capacitors, which serve as a memory bank.

A logic system directs or "times" the instrument to take readings at set wavelength points, corresponding to paired reference points on the spectral trace for, for example, protein. When the standard is replaced by a sample of ground material, some of the light is absorbed by the protein, and is not reflected back to the sensing head. The reduced amount of light that is reflected back to the sensing head is translated into energy and stored in a second series of capacitors. The storage process, or charging of the capacitors, takes about 15–25 s, which is the time of the test. The difference in the extent to which the two sets of capacitors become charged by the light energy is directly related to the amount of protein in the sample. This difference is amplified, translated into protein by means of an analog computer system inside the instrument, and displayed directly as protein on the digital panel meter.

There are three "channels," one each for oil, protein, and moisture, combined into a single rotating filter wheel. The reflectance energy of all three constituents is interrelated to a certain extent, and maximum accuracy is achieved by combining the contribution of all three channels in the measurement of any individual constituent. For example, the oil and moisture signals are used in the measurement of protein, as well as the protein signal.

The nature of the surface of the sample presented to the instrument influences the manner in which light is reflected from the surface, and the mathematics of the computation. Consequently, mean particle size, and particle size distribution are the most important factors affecting the accuracy of analysis. Sample preparation, grinding, and mixing of pulverized material must be carefully controlled. The most suitable grinder for the purpose is a high speed (at least 8500 rpm) hammer mill, or impeller-type grinder fitted with a 1.0-mm screen. Wheat ground in grinders of this type has a mean particle size of about 205 μm. The mean particle size of grains is influenced mainly by the texture of the grain, which is in turn affected by the type of wheat (i.e., hard red spring, soft white winter, etc.), by the growing environment, and season, and by such factors as weathering, tempering, and moisture level. The influence of most of these factors is minimized by using a grinder of the above type.

IRS instrumentation is capable of measuring constituents other than protein; moisture, oil, and starch are all associated with characteristic absorbances in the near infrared area of up to about 2400 nm. Furthermore, the instrumentation can be used to estimate any facet of grain quality that causes variance in the signal received by the instrument. For example, the influence of particle size has been utilized to furnish a means for the assessment of kernel hardness in wheat (31). For operations that quote protein results on the basis of dry matter, or constant moisture content, IRS instrumentation provides the means of simultaneous measurement of both protein and moisture on the same sample, thereby removing the possible error incurred as a result of moisture loss during sample preparation and grinding.

References

1. Kjeldahl, J.T. 1883. Z. Anal Chem. (Germany), 22, 366.
2. Wilforth, H. 1885. Chem. Zentralbl. Biol. (Germany), 16, 17.
3. Gunning, J.W. 1889. Z. Anal. Chem. 28, 188.
4. Winkler, L.W. 1913. A. Angew. Chem. 26, 231.
5. Bradstreet, R.B. 1965. The Kjeldahl method for

organic nitrogen. New York, U.S., Academic Press Inc.
6. American Association of Cereal Chemists. 1962. Approved methods, 7th ed. Minneapolis, U.S., American Association of Cereal Chemists.
7. Association of Official Analytical Chemists. 1970. Official methods of analysis of the Association of Official Agricultural Chemists, 11th ed. Benjamin Franklin Station, Washington, D.C., U.S., Association of Official Analytical Chemists.
8. Trevis, J.E. 1974. Cereal Sci. Today (U.S.), 19, 182.
9. Williams, P.C. 1974. Cereal Sci. Today (U.S.), 19, 280.
10. Bolleter, W.T., Bushman, C.J., and Tidwell, P.W. 1961. Anal. Chem. (U.S.), 33, 592.
11. Varley, J.A. 1966. Analyst, 91, 119.
12. Gehrke, C.W., Kaiser, F.E., and Ussary, J.P. 1968. J. Assoc. Off. Anal. Chem. (U.S.), 51, 200.
13. Gehrke, C.W., and Wall, L.L. 1971. J. Assoc. Off. Anal. Chem. (U.S.), 54, 187.
14. Crooke, W.M., and Simpson, W.E. 1971. J. Sci. Food Agric. (England), 22, 9.
15. Williams, P.C. 1964. Analyst, 89, 276.
16. Feinstein, L., and Hart, J.R. 1959. Cereal Chem. (U.S.), 36, 191.
17. Pinckney, A.J. 1961. Cereal Chem. (U.S.), 38, 501.
18. Jennings, A.C. 1961. Cereal Chem. (U.S.), 38, 467.
19. Williams, P.C., and McEwin, N.M. 1967. J. Sci. Food Agric. (England), 18, 184.
20. Johnson, R.M., and Craney, C.E. 1971. Cereal Chem. (U.S.), 48, 276.
21. Noll, J.S., Simmonds, D.H., and Bushuk, W. 1974. Cereal Chem. (U.S.), 51, 610.
22. Simmonds, D.H., and Ronalds, J.A. 1975. Bakers' Dig. (U.S.), 66, 36.
23. Udy, D.C. 1956. Cereal Chem. (U.S.), 33, 190.
24. Popineau, Y. 1975. Techniques des industries céréalières, No. 148, 10.
25. Gosbel, P. 1971, Flour Milling and Baking Research Association, Bull. No. 1, 18.
26. Johansson, A., Larsson, B., Tibell, G., and Ehrenberg, L. 1969. In New approaches to breeding for improved plant protein. Vienna, International Atomic Energy Agency, 169p.
27. Niemann, E.G., and Neumuller, D. 1973. In Nuclear techniques for seed protein improvement. Vienna, International Atomic Energy Agency, 339p.
28. Dohan, D.A., Standing, K.G., and Bushuk, W. 1976. Cereal Chem. (U.S.), 53, 91.
29. Rosenthal, R.D. 1971. Annual meeting, Kansas Association Wheat-growers, Hutchinson, Kansas, U.S.
30. Williams, P.C. 1975. Cereal Chem. (U.S.), 52, 561.
31. Williams, P.C. 1977. J. Sci. Food Agric. (England) (in press).

Calculation of the Nitrogen-to-Protein Conversion Factor

R. Tkachuk

The crude protein content of plant and other food materials is estimated by multiplying its total nitrogen (N) content by some factor, hence the term nitrogen-to-protein (or N:P) factor. The factor of 5.7, widely used for wheat, has its origins in the painstaking work of Osborne and Voorhees (1), who found that the average nitrogen content of the protein fractions they isolated was 17.54%. It was subsequently concluded that by determining the nitrogen content of any sample of wheat and multiplying the result by $100 \div 17.54$ (5.70), a good working estimate of the protein content would be realized. The principle has been extended to other commodities, as various workers have isolated and analyzed the proteins for nitrogen. Some of the more commonly used N:P factors are: wheat and wheaten products, 5.7; rice and rice products, 5.95; all other cereals, legumes, oilseeds, and forages, 6.25; and milk and dairy products, 6.38.

Proteins and peptides are polymers of amino acids (AA). The availability of accurate amino acid analysis now makes it possible to calculate the protein content of various materials more accurately. Dividing the protein content calculated from the AA composition by the nitrogen content of the sample leads to an accurate value for the N-to-P factor. When such calculations were carried out for some cereals and oilseeds (2, 4), N-to-P factors obtained were equal approximately to 5.5–5.7. The procedure of using 6.25 as a factor for all oilseeds, pulses, forages, and cereals (other than wheat and rice) is of doubtful validity. This factor was derived largely as a result of the analysis of proteins isolated from animal sources. Analytical studies made on a wide diversity of cereals and oilseeds have failed to identify any materials with a factor as high as 6.25 (2, 5).

Aside from the commercial aspect, the chief significance of the N:P factor concerns the nutritionist, who endeavours to formulate diets with a satisfactory balance of protein, carbohydrate, and other constituents from a mixture of

Table 1. Influence of protein level on N:P factor (Tkachuk, R., unpublished data).

	Pearl millet	Sorghum	Chick-pea	Teff
Protein (1)	9.5	7.0	19.3	10.1
N:P	5.36	5.58	5.53	5.31
Protein	17.1	14.6	23.5	13.4
N:P	5.49	5.62	5.44	5.42

Note: N × 5.7 dry basis.

Table 2. Influence of N:P factor on reported "protein" level.

	"Protein" at different % of N (Kjeldahl)				
	2	3	4	5	6% N
N:P factor					
5.2 (A)	10.4	15.6	20.8	26.0	31.2
5.7 (B)	11.4	17.1	22.8	28.5	34.2
6.0 (C)	12.0	18.0	24.0	30.0	36.0
6.25 (D)	12.50	18.75	25.0	31.25	37.5
Diff. D-A	2.10	3.15	4.20	5.25	6.30

materials, most of which differ in amino acid composition. Because amino acids differ both in their individual composition and in their distribution within proteins, most proteins themselves differ in nitrogen content. As a result, the N:P factor differs from crop to crop, and in most instances the factor of 6.25 will lead to spuriously high values for the "protein" figures. Diets and feed formulations based on these figures are unlikely to provide protein nutrition to conform with their apparent composition. A recent study on three cereals and a legume fed at different protein levels revealed that the N:P factor differed within a crop as a direct result of differences in amino-acid composition (Tkachuk, R., unpublished data). This study is summarized in Table 1. Not only did the N:P factors differ at different protein levels, but they differed in different directions.

The influence of the N:P factor on the "protein" content is illustrated in Table 2. The effect is mathematically most marked in materials with high nitrogen contents. Although legumes are regarded as reliable sources of high protein for human nutrition, this observation is of particular concern to breeders and nutritionists in the fields of legume production and utilization.

The most significant nitrogen-containing substances in protein synthesis and metabolism in nonruminant animals (including humans) are the amino acids, and certain amides, such as asparagine and glutamine. Consequently, the most logical method of arriving at a N:P factor is to determine the total amino acid and amide content of a commodity, and the respective distribution of amino acids and amide nitrogen. The total nitrogen content is then determined, and the ratio of total amino acids plus amides – total nitrogen in unit weight of sample gives the ratio N:amino acids plus amides, or the true N:P factor. The method of calculation is explained more fully in Appendix 1. Maximum recovery of amino acids is essential, and this is affected mainly by hydrolysis conditions (6). Accuracy is also improved by meticulous observation of such conditions as the relative moisture contents of the samples used, respectively, for total nitrogen and amino acid determinations, and by correction for the elements of water added during hydrolysis. In most mature plant material the amount of soluble nitrogen (usually referred to as "nonprotein" nitrogen) is only about 2–3%, and consists largely of free amino acids, simple peptides, and intermediate compounds in protein metabolism. In other words, practically all of the nitrogen in plant material can be accounted for in terms of some type of amino acid, or derivative thereof. As the recovery of nitrogen as amino acids after hydrolysis is rarely in excess of 96%, it is likely that N:P factors based on amino acid analysis are slightly low. A reduction in recovery of amino acids of 3% means that a true N:P factor of 5.8

Table 3. Protein data for some pulses compared with wheat (Dronzek, B.L.: unpublished data).

Parameter	Lentil *L. esculatum*	Bean *V. faba*	Pigeon pea *C. cajanus*	Chick-pea *C. arietinum*	Vetch *V. narbonensis*	Vetch *V. gallilea*	Wheat *T. aestivum*
N:P factor[a]	5.48	5.26	5.76	5.55	5.46	5.50	5.68
Mean protein[b]	28.1	28.1	23.0	21.9	29.1	26.4	14.1
High protein	36.4	34.2	25.8	26.0	33.3	—	—
Low protein	23.3	22.5	20.6	17.4	25.6	—	—
No. of samples	1688	511	40	2676	9	2	2
Lysine[c]	9.95	8.15	9.25	9.26	9.43	10.83	3.29
Histidine	5.23	4.45	6.88	4.64	4.27	4.58	3.82
Threonine	2.86	2.66	2.83	2.95	2.87	2.81	2.14
Valine	3.76	3.60	3.62	3.65	3.81	3.87	3.23
Methionine	0.34	0.35	0.61	0.73	0.35	0.33	0.77
Isoleucine	2.85	2.79	2.73	3.05	2.88	2.98	2.34
Leucine	5.14	5.09	5.34	5.45	5.11	5.42	4.70
Tyrosine	1.44	1.21	1.13	1.24	1.33	1.24	1.41
Phenylalanine	2.76	2.27	5.16	3.21	2.45	2.47	2.75

[a]Corrected to 98% recovery of amino acids.
[b]N × 5.7, dry basis. Standard error of check sample analysis (chick-pea) = 0.263%.
[c]Grams amino acids per 100 g protein.

will be reported as 5.62, for example.

Table 3 contains some details of the protein makeup of some common and less common legumes (6). Due partly to the absence of tryptophan and cystine figures, the recoveries were on the average rather low (89–90%), and for the purpose of this table, the figures were adjusted proportionally for each amino acid, to comply with a recovery of 98%. The data for wheat agrees well with literature values (2). Figures for total protein and some essential amino acids are included. All of the legumes provide an excellent source of lysine, compared with wheat. The legumes cited all provide better sources than wheat for all of the essential amino acids listed with the exception of methionine and tryptophan. The single sample of vetch *Vicia gallilea* was particularly rich in lysine. Lentil and bean (*Vicia faba*) were the richest sources of total protein.

The foregoing remarks underline the facts that (*a*) N:P factors vary widely between species of legume (as well as other commodities), and that, (*b*) none of the N:P factors are as high as 6.25. For all practical purposes, variation in the N:P factor is of no serious consequence, provided that the factor is quoted at the same time as the protein figures derived from it. The nutritionist and feed compositor can either compute food and feed mixes on the basis of total nitrogen, or convert, for example, N × 5.7 "protein" figures to N × 6.25 values to comply with practiced procedure in specific operations.

A worldwide campaign to standardize the N:P factor would in all probability spark a controversy that would endure until the turn of the century. However, were such a campaign to be waged, it would be more realistic to establish that the factor of 5.7 should serve as the standard N:P factor, for the reporting of protein content in all commodities of plant origin likely to be involved in the formulation of foods and feeds for human and other animals.

References

1. Osborne, T.B., and Voorhees, C.G. 1893. Am. Chem. J. (U.S.), 15, 392.
2. Tkachuk, R. 1969. Nitrogen-to-protein conversion factors for cereals and oilseed meals. Cereal Chem. (U.S.), 46, 419.
3. Heathcote, J.G. 1950. The quality of oats. Br. J. Nutr. (England), 4, 145.
4. Tkachuk, R. 1966. Note on the nitrogen-to-protein conversion factor for wheat flour. Cereal Chem. (U.S.), 43, 223.
5. Protein Calorie Advisory Group. 1974. Protein Advisory Group Statement No. 22.
6. Tkachuk, R., and Irvine, G.N. 1969. Amino acid compositions of cereals and oilseed meals. Cereal Chem. (U.S.), 46, 206.

Appendix Table 1. Calculation of N:P factor (includes typical values for series I for beans). Original protein content of sample = 23.7% (N × 5.7 dry basis).

AA	G AA N/ 100 g total N, I	K_{AA} (N × 5.7)	K_{AA} (N × 6.25)	G AA residues/ 100 g sample II
Tryptophan	0.482	0.8575	0.9400	0.562
Lysine	8.343	1.2450	1.3651	6.702
Histidine	4.583	1.7455	1.9139	2.626
NH_3	10.683	4.6877	5.1403	2.279
Arginine	21.584	2.0424	2.2395	10.568
Aspartic acid	8.123	0.6932	0.7601	11.718
Threonine	2.738	0.7892	0.8650	3.469
Serine	3.518	0.9373	1.0278	3.753
Glutamic acid	10.001	0.6180	0.6777	16.183
Proline	3.270	0.8212	0.9005	3.982
Glycine	5.319	1.3987	1.5337	3.803
Alanine	4.313	1.1222	1.2304	3.843
Valine	3.601	0.8052	0.8829	4.472
Cystine	0.617	0.7186	0.7877	0.859
Methionine	0.342	0.6083	0.6669	0.562
Isoleucine	2.767	0.7048	0.7728	3.926
Leucine	5.001	0.7084	0.7728	7.096
Tyrosine	1.477	0.4896	0.5368	3.017
Phenylalanine	2.330	0.5425	0.5948	4.295
Total series II				93.715

Total II ÷ (100 ÷ 23.7) = 93.715 ÷ 4.2194 = 22.2105 = III

Total III ÷ % N in sample = 22.2105 ÷ 4.1579 = 5.34 = N:P factor

Appendix

Calculation of N:P Factor

1. The amino acid (AA) analyzer should be programed to yield the data for grams AA nitrogen (N) per 100 g of total N, or the equivalent = I.

2. Derive the corresponding figures for grams AA residues per 100 g protein. In proteins the AAs are linked by peptide bonds. The elements of water are added to each molecule during hydrolysis, so that the recovery of AAs should be greater than 100%. To get a true picture of the relationship of N to AAs, the AA figures from hydrolyzates must be corrected to the original residue form in which they exist in proteins.

Divide the figures from series I by an individual factor, K_{AA} for each AA. The K_{AA} constants depend on the original N:P factor used in reporting the "protein" content of the sample, and the molecular weight and nitrogen content of the individual AAs.

Grams AA residues/100 g protein = II.

The K_{AA} constants for all common AAs are listed in Appendix Table 1 for N × 5.7 and N × 6.25.

3. Total series II and divide by (100 ÷ % protein in original sample), to arrive at % AA residues in original sample = III.

4. Divide III by % N in original sample to derive the ratio of total N : total AA residues. This is the true N:P factor. A typical calculation follows in Appendix Table 1.

5. Ensure that all AA, "protein" and total N results are reported on the same moisture basis (preferably moisture-free) to avoid serious errors in computation of N:P factor.

Biological Assays for Protein Quality

J.M. McLaughlan

It has been known for more than a century that proteins differ in their nutritional value for animals. Gelatin, in particular, was shown to be much inferior to most other proteins. The concept of "biological value" and the first real protein methodology for nutritional evaluation was proposed by Thomas in 1909. Although the method, applied and modified by Mitchell (1), is theoretically sound, it is too laborious and time consuming for ordinary use. In 1919, Osborne et al. (2) introduced a simple rat growth assay called protein efficiency ratio (PER); this procedure, with some modification, is now probably the most widely used method for evaluating protein quality. PER is influenced by several factors, but the major criticism of the method is that individual PER values are not proportional; a PER of 2.0 is not twice as good as a PER of 1.0. This problem arises because PER does not make allowance for protein utilized for maintenance purposes.

Bender and Miller (3, 4) introduced an assay for net protein utilization (NPU) referred to as the carcass analysis method, which does allow for protein required for maintenance. NPU is widely used, but it is also laborious because it is necessary to measure the nitrogen content of rat carcasses. Bender and Doell (5) later proposed a simple modification of NPU in which body weight rather than body nitrogen was measured; the method was called net protein ratio (NPR). This is the same as PER but adding the weight *loss* of the nonprotein group to the weight gain allows calculation of values from poor-quality proteins that do not support growth. Several groups of workers have shown that changes in body weight accurately reflect changes in body nitrogen in short-term (i.e., 10–14 days) tests (6, 7). Body weight can be determined readily and with less error than body nitrogen; therefore, the use of body weight instead of body nitrogen reduced the variability of assays.

Until recently, PER was the method of choice in North America, whereas NPU was more commonly used in Europe. In 1965, Hegsted and Chang (8, 9) proposed a multidose slope-ratio assay. This was a modification of the nitrogen balance index devised by Allison and Anderson (10), which has been used extensively by Bressani and co-workers (11). Hegsted and Chang (8, 9) claimed that the slope ratio had the characteristics of a good bioassay — provided that the body weight response was linear over the range of protein levels involved in the estimation of the slope. A reference standard protein was included in each assay and the slopes of the response lines for reference and test proteins were expressed as ratios. For a valid slope-ratio assay the response

lines should be linear and meet at a common point on the Y-axis, which should be the weight loss of the rats fed a nonprotein diet. Experience with the slope-ratio method has shown that this is not usually true, particularly for lysine-deficient proteins (12, 13). Consideration is being given to using the true slopes of the response lines and not including the group of rats fed the nonprotein diet in the calculation of the slopes (14). This modified slope-ratio assay is called relative protein value (RPV).

The relations between PER, NPR, and multi-dose slope-ratio assays are illustrated in Fig. 1. Weight changes with four doses of protein are plotted. The response line "d" shows the ideal relation. PER is a one-dose assay; NPR is a two-dose assay; and slope ratio is multi-dose.

$$\text{PER} = \frac{\text{weight gain of test group (g)}}{\text{protein consumed (g)}}$$

$$\text{PER at weight gain of 52.5 g} = \frac{52.5}{10.5} = 5.0$$

$$\text{PER at weight gain of 20 g} = \frac{20}{5.25} = 3.81$$

$$\text{NPR} = \frac{\text{weight gain of test group (g)} + \text{weight loss of nonprotein group (g)}}{\text{protein consumed (g)}}$$

$$\text{NPR at weight gain of 52.5 g} = \frac{a+b}{c} = \frac{52.5+12.5}{10.5} = 6.19$$

$$\text{NPR at weight gain of 20 g} = \frac{20+12.5}{5.25} = 6.19$$

The slope of response line "d" as used in the slope-ratio assays

$$= \frac{a+b}{c} = \frac{52.5+12.5}{10.5} = 6.19$$

The most obvious point is that the NPR value is the slope of the response line. Consequently NPR and slope-ratio assay give identical results if the assay is perfect (i.e., all doses falling on the straight-line response). With PER there is no relation between PERs at different protein levels. In Fig. 1 the PER is zero with a protein intake of 2.0 g.

PER is a one-dose assay carried out at 9–10% protein level in the diet. NPR is a two-dose assay (8–10% and zero protein). The slope of the

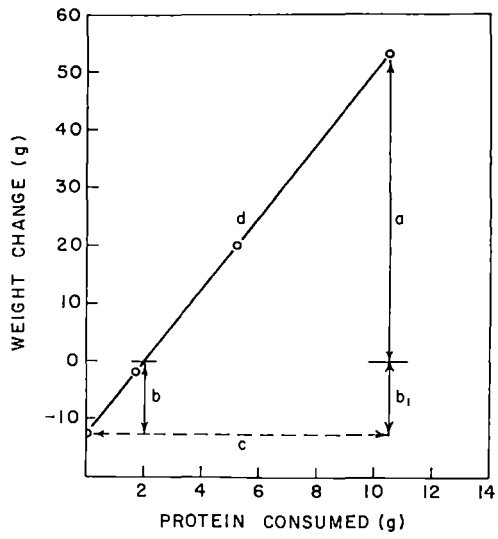


Fig. 1. Theoretical "ideal" relation between weight change of rats fed four levels of dietary protein and protein consumed.

response in the multidose assay is influenced by the two terminal doses. It is not surprising, therefore, that there is generally good agreement among protein quality methods. Nevertheless, there are significant differences between methods for low quality proteins — this is illustrated in Table 1. Each protein quality method is expressed on a scale of 1 to 100, and lactalbumin is arbitrarily set at 100. The customary protein quality assay diet contains 10% lipid. When the diet contained 20% lipid the rats ate less food; the lower food intake affected the PER assay to a greater extent than the other two methods. A poor protein such as pea had a low PER that increased fourfold by supplementation with methionine; the other two methods merely showed a doubling of protein quality. Lysine-deficient protein such as wheat gluten had a very low value by PER; NPR gave a relatively high value and the result by the RPV assay was intermediate. The last three samples, bread, beans, and bread plus beans, illustrate supplementary effects between proteins. Bread and beans individually had low protein quality values, but the mixture of the two proteins had a much better value than either alone. Bread is deficient in lysine but has a relative abundance of methionine plus cystine; the reverse is true for beans. Supplementary effects can be readily predicted using amino acid score (chemical score).

The use of several different methods for estimating protein quality means that there is no

unanimity as to which is the most suitable method for routine biological evaluation of food proteins. Each of the methods discussed has serious shortcomings. Hegsted and co-workers (8, 9) have critized one- and two-dose assays (BV,[1] PER, NPU, and NPR) and have pointed out the theoretical advantages of a multidose assay, but there are problems with lysine-deficient proteins (12, 13) for the slope-ratio assay.

The question arises, why does the biological evaluation of protein quality with rats present such problems? The difficulties may relate to what we mean by "protein quality." Some workers consider protein quality to be a fixed entity, an inherent property of the protein, but others consider it to be a variable characteristic. It is generally agreed that protein quality for the rat depends largely, but not entirely, on the concentration of the limiting amino acid and the rat's requirement for that amino acid. As the rat's requirements for specific amino acids probably differ for maintenance and growth purposes (12, 14), protein quality may differ at different levels of protein intake (i.e., levels required for maintenance and for growth). In view of the factors involved in protein quality (different limiting amino acids in different proteins and the effect of different protein levels), it is probably impossible to devise a fully satisfactory assay for protein quality. Nevertheless, even PER is a useful measure, particularly for good-quality proteins. A recent collaborative assay indicated that both NPR and slope-ratio are superior to PER.

It seems likely that either the NPR or the RPV method will replace PER as the most widely applied method for estimating protein quality (15). The RPV assay producing ideal data yields exactly the same value as NPR. However, the NPR method gives higher values than the RPV method for lysine-deficient proteins. At present, it is not possible to decide which result is correct. One problem with the slope assay is the need for larger amounts of test protein due to multiple doses, and this may limit its usefulness in plant breeding programs.

Amino acid score (chemical score), which is based on the amino acid composition of the protein, is a rapid, useful method for estimating protein quality. This approach was originally proposed by Block and Mitchell (16) who used the essential amino acid pattern of whole egg as a reference. Egg protein was presumed to have an ideal amino acid pattern and it gave the highest protein quality measures in both man and the growing rat. Each of the essential amino acids in the test protein was expressed as a percentage of the amount of that amino acid in egg. Chemical score of a test protein was the value for the amino acid in greatest deficit relative to egg protein. Chemical score indicated both the limiting amino acid and the relative nutritive value of the protein. Although several studies have demonstrated the value of chemical score other studies have shown that the sulfur-containing amino acids (methionine and cystine) are too high in egg for a good reference protein (17).

Table 1. Comparison of values for several proteins by three methods (lactalbumin = 100).

Protein source	PER	NPR	RPV
Lactalbumin	100	100	100
Lactalbumin (20% lipid)	82	94	95
Pea	19	47	36
Pea + methionine 1	58	69	68
Pea + methionine 2	85	86	83
Soya flour	68	73	74
Wheat gluten	5	37	23
Bread	17	38	—
Beans	20	45	—
Bread + beans	54	64	—

Several other amino acid scoring patterns have been suggested, but the latest and probably most appropriate pattern is based on human amino acid requirements (18). Despite considerable differences in the amino acid content of the various scoring patterns, cereals are always deficient in lysine, and legumes are limiting in methionine plus cystine (a few may be equally limiting in tryptophan). Several studies have shown a high degree of correlation between lysine content and PER of a wide variety of cereals.

In using amino acid score, the assumption is made that amino acids are fully available — this isn't always true. Digestibility, particularly for beans is a real problem (19). Amino acid score gives an excellent picture of the potential nutritive value of a protein — but the actual value may be lower due to incomplete availability of amino acids. If amino acids were completely available there wouldn't be any need for a bioassay for protein quality.

[1]BV, biological value.

References

1. Mitchell, H.H. 1923-24. J. Biol. Chem. (U.S.), 58, 873.
2. Osborne, T.B., Mendel, L.B., and Ferry, E.L. 1919. J. Biol. Chem. (U.S.), 37, 223.
3. Bender, A.E., and Miller, D.S. 1953. Biochem. J. (England), 53, vii.
4. Miller, D.S., and Bender, A.E. 1955. Br. J. Nutr. (England), 9, 383.
5. Bender, A.E., and Doell, B.H. 1957. Br. J. Nutr. (England), 11, 140.
6. Donoso, G. and Yáñez, E. 1962. Nutr. Bromatol. Toxicol. 1, 37.
7. Hegsted, D.M., Neff, R., and Worcester, J. 1968. J. Agric. Food Chem. (U.S.), 16, 190.
8. Hegsted, D.M., and Chang, Y. 1965. J. Nutr. (U.S.), 85, 159.
9. Hegsted, D.M., and Chang, Y. 1965. J. Nutr. (U.S.), 87, 19.
10. Allison, J.B., and Anderson, J.A. 1945. J. Nutr. (U.S.), 29, 413.
11. Bressani, R., Viteri, F., and Elías, L.G. 1973. Proteins in human nutrition. In Porter, J.W.G., and Rolls, B.A., ed., London and New York, Academic Press Inc.
12. McLaughlan, J.M., and Campbell, J.A. 1969. Mammalian protein metabolism, vol. 3. In Munro, H.N., ed., New York, U.S., Academic Press Inc.
13. Yáñez, E., and McLaughlan, J.M. 1970. Can. J. Physiol. Pharmacol. (Canada), 48, 188.
14. Said, A.K., and Hegsted, D.M. 1970. J. Nutr. (U.S.), 100, 1363.
15. Protein Advisory Group. 1975. PAG Bulletin, vol. V, no. 2, June 1975. New York, U.S., Protein-Calorie Advisory Group of the United Nations System.
16. Block, R.J., and Mitchell, H.H. 1946. Nutr. Abstr. Rev. (England), 16, 249.
17. McLaughlan, J.M., Rogers, C.G., Chapman, D.G., and Campbell, J.A. 1959. Can. J. Biochem. Physiol. (Canada), 37, 1293.
18. Food and Agriculture Organization of the United Nations/World Health Organization. 1973. Energy and protein requirements: report of a joint FAO/WHO *ad hoc* expert committee. FAO nutrition reports series No. 52. Rome, Italy, Food and Agriculture Organization.
19. Bressani, R., and Elías, L.G. 1977. (See chapter 5 page 61 of this publication.)

Criteria for Cooking Quality and Acceptability of Cowpeas

Florence E. Dovlo

Because of limitations in the amino acid composition of proteins in leguminous grains, such grains have poor nutritional quality. For this reason, there is currently a growing concern for improvement of the quality of protein in these relatively inexpensive food legumes to increase their nutritional contribution to diets of low-income groups in particular, and also to make cowpea meals suitable for infant feeding.

Legume breeders are urged to include improvement of the amino acid profiles of legume proteins in their breeding programs, and to eliminate certain undesirable characteristics of food legumes, as well as identifying high-yield genotypes that are also weevil-resistant. It is equally important for breeders to take into account the cooking quality and consumer acceptance of the new cowpea cultivars.

Cowpeas (*Vigna unguiculata*) are among the most widely used of the food legumes. Cowpeas are of different sizes, shapes, and colour, and are used in a great variety of ways (1). In addition to their visual characteristics, cowpeas have intrinsic differences in their cooking quality, texture, and flavour. Consumer studies show that consumers have particular preferences for various uses of the different types of cowpea. It is therefore essential that legume breeders be cognizant of these preferences (2). For consumer acceptance it is important that the type of cowpea be fast-cooking and capable of doubling in quantity. Ability of the grains to bind is another desired quality for certain types of dishes. Taste and flavour are important factors for consumer acceptance.

For plain cooking, and for combinations with cereals, the brown type of cowpea is preferred and generally used to avoid monotony in the colour of the dish. The bright maroon-red cowpeas are usually preferred for stew, with the grains remaining firm after cooking.

Cowpeas are also processed into paste or flour and used in making certain cowpea dishes that are fried or steamed. In this process, the grains are first dehulled, then ground to make the paste, or alternatively, dried and ground into flour. For this operation, ease of soaking and dehulling are essential. The cream-coloured cowpea has been found easiest to dehull (2).

For some processed cowpea dishes, the paste or flour is whipped before use. In this usage, desirable characteristics of the flour or paste are

its binding quality and capacity to rise.

Assessing Cowpea Characteristics

Dehulling test

This is a simple household test. Note the type of seed coat i.e., shiny, wrinkled, etc., of the dry cowpea sample. Soak a few cowpeas in water at room temperature for a brief period (10 min). Rub the soaked seeds between the fingers and note the effort needed to remove the coat or testa. Relate the ease of dehulling to the nature of the sample's dry surface.

Estimation of cooking quality

Weigh into individual perforated containers approximately 10 g of cowpea samples. Immerse each sample in a litre of boiling water. Remove from water, drain, and record weight increase at 5-min intervals. Cooking time is the time at which the grains become soft and cease to gain weight. Repeat the test.

Softness can be determined manually by simply mashing the cooked grains between the fingers or with a spoon. Water uptake or swelling capacity can be correlated with cooking time. Note changes in the colour of the grains after cooking. The grains initially absorb water linearly with time taken, and swelling capacity is directly related to water absorption (3).

Alternatively, samples can be screened by boiling the grains for one standard time and ranked for softness. A more scientific and accurate measurement of the cooking quality of cowpea can be done mechanically by using texture-measuring instruments, such as a texturmeter or a shear compression cell.

Breeders should take note of the influence of certain salts on the cooking time of cowpea. Phytin, calcium, magnesium, and free pectin content of beans affect their cooking quality (4).

Functional properties

Functional properties of the flour or paste such as fat absorption, foaming characteristics, emulsification, water absorption, and nitrogen solubility are important specific product requirements.

Fat absorption — Weigh a 2-g sample of flour into a 15-ml conical centrifuge tube and add 5 ml of edible oil. Stir the mixture thoroughly with a brass wire. Allow the tubes to stand for 30 min, then centrifuge at 2100G for 25 min. Read the volume of supernatant oil and calculate the percentage oil absorbed (5).

$$\% \text{ oil absorbed} = \frac{X - Y}{X} \times \frac{100}{1}$$

where X = initial oil used and Y = supernatant oil.

Water absorption — Weigh a 2.5-g sample of flour into a centrifuge tube. Add 15 ml of water. Agitate the tube by hand until the sample is dispersed in water. Place a stopper on the centrifuge tube and shake for 1 h at No. 10 speed on a Burrel shaker. Centrifuge at 1200G for 25 min. Remove the supernatant by pipette. Determine the water retained by weighing and express as a percentage of the original sample weight (6).

$$\% \text{ water retained} = \frac{Y - X}{X} \times 100$$

where X = initial weight, Y = weight after absorption, and Y − X = weight of water retained.

Foaming quality — Weigh a 6-g sample of flour into 60 ml water. Whip in a small bowl with mixer at high speed for varying lengths of time. Transfer the resulting foam to a 250-ml graduated cylinder and measure the initial foam volume. Evaluate the foam stability by measuring the foam value after standing for intervals of 5, 10, 30, 60, and 120 min (7).

Emulsification — Weigh a 7-g sample of flour and disperse in 100 ml of water and 100 ml groundnut oil. Using a blender, emulsify the 200-ml mixture at high speed for 1 min. Divide 200 ml of the emulsion into four 50 ml centrifuge tubes and centrifuge at 1300G for 5 min (8).

$$\% \text{ emulsified} = \frac{X}{Y} \times \frac{100}{1}$$

where X = height of emulsified layer and Y = height of whole layer. Measure the emulsion stability by heating the emulsion, prepared as above, at 80 °C for 30 min. Cool under tap water and centrifuge at 1300G for 5 min. The emulsion stability can then be expressed as a percentage.

$$\% \text{ emulsion stability} = \frac{X}{Y} \times \frac{100}{1}$$

where X = height of remaining emulsified layer and Y = height of whole emulsion layer in tube.

Determination of nitrogen solubility index — Using a modification of Lyman's method (9), weigh a 1-g sample 60 mesh screen) into a 300-ml Erlenmeyer flask with four glass beads. Add 100 ml of distilled water and shake the flask rapidly on a mechanical shaker in an incubator or water bath at 37 °C for 1 h.

Centrifuge the material at 3000 rpm for 5 min. Filter through coarse filter paper. Pipette 50 ml of supernantant into a Kjeldahl flask. Add 2 ml of conc. H_2SO_4 and evaporate almost to dryness, on the digestion rack. Determine nitrogen by

Kjeldahl method.

$$\% \text{ w/w nitrogen} = \frac{\text{titre} \times \text{normality} \times 0.014 \times 100}{\text{wt of sample}}.$$

$$\text{Nitrogen solubility index} = \frac{\% \text{ soluble nitrogen} \times 100}{\% \text{ total nitrogen of sample}}.$$

Sensory Evaluation

In addition to mechanical assessment of the acceptability of a pulse, taste-panel evaluation is also essential.

Samples of a new cultivar of cowpea should be evaluated by a well-selected panel. For visual characteristics, the new cultivar and samples of other local types should be coded and ranked in order of preference for grain colour, size, and eye type. Cooked samples should be graded for taste and texture.

Selection of panelists

Panel members may be selected from research staff or personnel from the office. It is important to select people in good health. Anyone with a cold should not take part.

Panelists should have a high sensitivity and degree of personal integrity and intellectual curiosity, and should be willing to spend time on the evaluation. Out of several trials, a group of reliable panelists can be formed. The number of panelists should be 8–10. The services of a well-qualified home economist may be used to undertake the cooking tests and studies of the functional properties, and to conduct the sensory evaluation.

Sample Evaluation Forms

1. **Visual Characteristics**
   Rank these cowpea samples in order of preference for colour, grain size, and eye type.

Choice	Colour	Grain size	Eye type
1st			
2nd			
3rd			
4th			

   Any comments:

2. **Samples Cooked for Standard Period**
   Evaluate these samples for softness (texture).

Code No.	Too soft	Slightly soft	Just right	Slightly tough	Very tough	Not cooked
e.g., 058						

   Any comments:

3. **Test for Taste**
   Taste these samples of cowpea and check your preference.

Code No.	Like very much	Like slightly	Don't like
e.g., 049			

   Any comments:

References

1. Dovlo, F.E., Williams, C.E., and Zoaka, L. 1976. Cowpeas — Home preparation and use in West Africa. In Graham, M., ed., Ottawa, International Development Research Centre, IDRC-055e.
2. Dovlo, F.E. 1975. Consumer preference for cowpea varieties in the Volta region of Ghana. Accra, Ghana, Food Research Institute.
3. International Institute of Tropical Agriculture, 1974. International Institute of Tropical Agriculture, Annual Report 1974, Ibadan, Nigeria, 74p.
4. Muller, F.M. 1967. Cooking quality of pulses. J. Sci. Food Agric. (England), 18, 292.
5. Shuey, W.C., Rask, P.S. et al. 1963. Measuring the oil binding characteristics of flour. Cereal Chem. (U.S.), 40(1), 71–77.
6. Sosulski, F.W. 1962. The centrifuge method for determining flour absorption in hard red Spring wheats. Cereal Chem. (U.S.), 39, 344.
7. Saterlee, L.D., Bembers, M., and Kendrich, J.G. 1975. J. Food Sci. (U.S.), 40(1), 81–84.
8. Mori, H. et al. 1972. Agric. Biol. Chem. (Japan), 36(5), 719–727.
9. Lyman, W.-Y. C., and Couch, J.R. 1953. J. Nutr. (U.S.), 49, 679.
10. Larmond, E. 1970. Methods for sensory evaluation of food.
11. Summer, A.K., Nielsen, M.A. et al. 1974/75. Food legume utilization. Progress Reports Nos. 1, 2 & 3, 1974/75 Saskatchewan, Canada.

Problems of Nutritional Quality of Pigeon Pea and Chick-pea and Prospects of Research

J.H. Hulse

Because of the extent of the relevant published literature, the principal difficulty in preparing this paper has been to decide what to omit rather than what to include. Many worthy papers are missing from the list of references because of the limits of space and time. However copies of all those cited are available at the International Development Research Centre (IDRC) and will be made available to anyone seriously interested.

Though the two food legumes under discussion appear under a variety of names, throughout this paper "*Cicer arietinum*" will be referred to as chick-pea and "*Cajanus cajan*" as pigeon pea.

Since the volume of published scientific literature that describes chick-pea appears much larger than that on pigeon pea, the subsequent text may appear somewhat unbalanced in relative content.

Food legumes can be described as potentially the most valuable yet probably the least developed of the naturally occurring sources of food protein. The nutritional value of legumes was recognized by the author of the book of Daniel (Daniel I verse 12) who wrote:

> "Prove thy servants I beseech thee ten days; and let them give us pulse to eat and water to drink then let our countenances be looked upon before thee and the countenances of the children that eat of the portion of the King's meat.... At the end of 10 days their countenances appeared fairer and fatter in flesh than all the children which did eat the portion of the King's meat. Thus Melzar took away the portion of their meat and the wine that they should drink and gave them pulse."

Chick-pea appears to have originated in the fertile crescent of the Mediterranean. Though Arnon (1)[1] suggests the crop had its earliest origins in the Himalayas, recipes including chick-pea are to be found in *De Re Coquinaria*, one of the earliest known cookbooks, which was written by the Roman gourmet Apicius, in *The Deipnosophists* by Athenaeus, and by Pliny the Elder in his *Historia Naturalis*. Athenaeus describes dishes containing boiled and roasted chick-peas and the use of the tender and mature seeds in several desserts. Pliny recommends chick-pea as a diuretic, to stimulate lactation, and also to prevent skin diseases.

Although the results of archaeological excavations around the Mediterranean appear to have firmly established the origin of chick-pea, the birthplace of the pigeon pea, so called because it is said to be a favourite of the wild pigeon, appears less certain. Watt (2) reported that pigeon pea grew wild in China and in the countries of Indochina. De Candolle (3) reported that pigeon pea was to be found in Africa from Zanzibar to the coast of Guinea and at about the same time pigeon pea was said to be growing wild in the region of the Upper Nile.

From earliest times the food legumes in general and chick-pea in particular have been stigmatized as the food of the poor and even today in Latin America it is descriptive of a poor man to state that he is "counting his garbanzos."

One could write a major work on the various ways in which chick-pea and pigeon pea are cooked and eaten in different parts of the world. They may be eaten raw as immature green seeds, or as cooked or milled dried pulses. The seeds may be parched, or roasted over open fires, in metal pans or on hot sand.

In India probably more than 75% of the chick-peas produced are milled to produce dhal. In several Middle Eastern countries milled chick-peas are mixed with wheat and other cereal flours to make a variety of fermented breads and sweet

Table 1. World production (1972) of major legumes.

Source[a]	'000 metric tonnes
Soybeans	52712
Groundnuts	16532
Phaseolus vulgaris	11010
Pisum sativum	10731
Chick-pea	7415
Vicia faba	5286
Pigeon pea	1648
Cowpea	1146
World total	106480

[a]Source: see ref. 34.

[1]In the interest of brevity only a comparatively few relevant references have been cited in the text. An additional longer reference list is provided, and copies of all of the publications quoted are available at IDRC in Ottawa.

Table 2. Chick-pea and pigeon pea production 1972.

	'000 metric tonnes		
	Chick-pea	Pigeon pea	All major pulses[a]
Developed countries	132		8713
Latin America	186	34	4345
Near East	235		788
Asia and Far East	6530	1548	19854
Africa	332	66	3536
All developing countries	7283	1648	28523
World	7415	1648	37236

[a]*Phaseolus vulgaris, Vicia faba, Pisum sativum, Cicer arietinum, Cajanus cajan, Vigna* spp.

Table 3. Percentage change in population and legume production 1952–1972 (based on FAO statistics).[a]

	Population	Total food	Food per capita	Chick-pea
Developed countries	+22	+60	+32	−42
Latin America	+62	+65	+ 2	+78
Near East	+57	+65	+ 2	+64
Asia and Far East	+51	+65	+ 9	+40
Africa	+52	+47	−3	+55
All developing countries	+53	+62	+ 6	+42
World	+40	+61	+15	+38

[a]Source: see ref. 3.

breads, in addition to being combined with meat, vegetables and (or) spices in many very delicious dishes.

The total world production of the major legumes is given in Table 1. If we exclude soya beans and groundnuts, chick-pea falls third and pigeon pea fifth in order of production. Table 2 presents production data for 1972 by major regions and it can be seen that Asia and the Far East provide roughly 90% of both the world's chick-pea and pigeon pea production. Mauritania (24 g per person/day) is the largest per capita producer of chick-pea, with Togo and India (20 g per person/day) in second place. The Dominican Republic (15 g per person/day) is the largest and India (8 g per person/day) the second largest per capita producer of pigeon pea.

In terms of total production, India is the largest producer of both pigeon pea and chick-pea. According to Swaminathan and Jain (4) chick-pea represented 51.0% and pigeon pea 11.2% of India's total pulse production of approximately 11.7 million tons in 1969–70.

Table 3 presents the percentage change in (*a*) population, (*b*) total food production, (*c*) food per capita, and (*d*) chick-pea production for the world as a whole and for the principal developing regions of the world.

In the world as a whole, the population has increased by roughly 40% while chick-pea production has increased by 38%. In Latin America and the Near East chick-pea production has risen faster than the population. In Asia legume production in general, and chick-pea production in particular, have fallen markedly behind the rate of population increase and at a noticeably lower rate than the percentage increase in total food production.

Cereal protein and legume protein are nutritionally complementary, those amino acids that are deficient in the one being generally adequate in the other. As a broad generalization, a diet in which protein derived from cereals and protein derived from food legumes are in approximately a 70:30 ratio comes very close to nutritional adequacy.

According to FAO (5) based on total production statistics, only in Latin America does the ratio of cereal protein to legume protein approach a 70:30 ratio. In Africa and the Near East

Table 4. Analyses of whole and dehusked chick-pea.[a]

	Whole	Dehusked
Ether extract, %	3.9–6.2	4.6–6.9
N × 6.25%	20.8–25.9	25.3–28.9
Soluble carbohydrate, %	60–63	63–65
Crude fiber, %	8.0–8.7	1.0–1.5
Ash, %	3.0–3.3	2.5–2.9

[a]Source: see ref. 5.

Table 5. Analyses of chick-pea and pigeon pea by micro-Kjeldahl and Udy (ICRISAT results).

	Mean	Variance	Range
Chick-pea			
Udy	23.77	0.79	22.58–26.56
Micro-Kjeldahl	23.47	0.78	21.5–25.13
Pigeon pea			
Udy	21.44	0.61	19.26–23.17
Micro-Kjeldahl	21.04	1.02	18.1–23.31
Pigeon pea with seed coat removed			
Udy	24.87	0.89	23.64–26.24
Micro-Kjeldahl	25.25	1.52	23.52–27.58

the ratio is 75 cereal to 25 legume, whereas in Southeast Asia it is closer to a 90:10 ratio. World cereal production is increasing at a much faster rate than world legume production: consequently, the need to increase legume production on a worldwide basis and in particular in South and Southeast Asia must be regarded as a matter of vital urgency.

In the FAO publication *Amino Acid Content of Foods* (6) the average protein content of chick-pea (N × 6.25) is quoted as 20.1 and of pigeon pea (N × 6.25) as 20.9%.

Swaminathan and Jain (4) give the results from 16 varieties of chick-pea grown at 12 locations and 11 varieties of pigeon pea grown at 5 locations. They state the range of protein in chick-pea from 12.4 to 28.1 with a mean of 19.5 and in pigeon pea from 18.5 to 26.3% with a mean of 21.5.

Because many of the protein contents quoted in the literature are based on a wide variety of sources and various methods of analysis, they are not all readily comparable. Many may prove to be of little practical value to the plant breeder, since in comparatively few instances are the identity and origin of the samples analyzed clearly defined.

Argikar (7) reports protein contents (N × 6.25 on a moisture-free basis) from 17.5 to 27.9, the results being from different strains grown at different localities. Also in the same publication the author quotes analytical results from a range of eight different strains of chick-pea (see Table 4).

In the same publication it is suggested that soil condition may influence protein content in that of chick-pea, which ranged in protein content from 17.5 to 27.9%; the highest tended to be those strains grown in alluvial soils. Protein contents of those grown on black cotton soils were 17.5, 17.9, 19.7, 20.0, 22.0 and 26.3%. Those grown on alluvial soils were 22.7, 26.3, 27.7, and 27.9%. However, because in this case the strains were all different, it may be difficult to isolate environmental from genetic influences.

Lal et al. (8) analyzed 47 pure strains of chick-pea, 24 of which were described as Common and 23 Kabuli. Common strains varied in protein content from a low of 17.38 (strain BR17 from Bihar) to a high of 23.8 (strain G2 from Madhya Pradesh). The Kabuli strains ranged from a low of 19.65 (strain NP7 — Indian Agricultural Research Institute (IARI) to a high of 25.41 (strain Rabat from the Punjab). The authors believe that Kabuli strains are genuinely and significantly higher in protein, ether extract, and

Table 6. Protein, lysine, and methionine content of chick-pea.

%	Part of cotyledon	
	Inner	Outer
Wt	25.1	64.7
Crude protein	19.4	25.7
Lysine	1.23	1.79
Methionine	0.21	0.29

iron content. They claim the Common strains to be higher in crude fiber and calcium.

Chandra and Arora (9) analyzed 40 varieties of chick-pea, all of which were grown in the Punjab. They identified four high protein varieties (N × 6.25 = 29.8%). The names and sources of origin of the four high protein varieties were: (*a*) Algeria 3444 — Algeria; (*b*) Frontier 8A — Pakistan; (*c*) Gram Cross A — India; (*d*) Gadag S2 — India. The low protein varieties (N × 6.25 = 18.4%) were (*a*) Tehran 29 and (*b*) Ahmedabad S1.

Dr Hugh Doggett kindly supplied us with the results of protein nitrogen analyses carried out at the International Crops Reseach Institute for the Semi-Arid Tropics (ICRISAT) by (*a*) micro-Kjeldahl, and (*b*) the Udy dye-binding method on 29 samples of chick-pea, 85 samples of pigeon pea, and 14 dehusked samples of pigeon pea. The results are given in Table 5.

In IARI (10) the yields and protein contents of "high yielding" varieties of chick-pea and pigeon pea are quoted. The chick-pea varieties range in protein content from 22.4 to 24.7%, the yields from 917 to 1053 kg/ha, and the protein yields from 215.5 to 260 kg/ha. Pigeon pea ranges were: protein 20.7–21.1%; yield 1250–1682 kg/ha, protein yield 259–340 hg/ha.

Zimmerman et al. (11) fractionated, by hand scalping, the cotyledons of random samples of chick-pea into an inner and outer portion and analyzed for protein, lysine, and methionine (see Tables 6 and 7). The weight (%) represents the proportions of the whole seed. The balance of 10.2% represents the combined weight of hulls and embryo.

Many authors have published the results of their analyses for essential amino acids in chick-pea and pigeon pea. A selection of these results are presented in Tables 8 and 9.

Hanuamantha Rao and Subramania (12) (see column A of Tables 8 and 9) quote the range of results from 15 different papers. They also quote (column G) results that they determined using paper chromatography. In columns E and F are the range of results from various sources reviewed by FAO (E) and the average of the same FAO data (F). The variability of results among different authors is readily apparent. To what extent the variation reflects genuine differences between samples, and to what extent it is attributable to inconsistencies in methodology and experimental error it is difficult to say. In any event, very few of the results quoted will be of great help to plant breeders, since rarely do the authors state precisely the nature, biological history, and source of the materials analyzed. The column labeled "WHO" in both tables quote the World Health Organization's recommended reference amino acid pattern, what might loosely be described as "an ideal proportion of essential amino acids."

Table 10 presents Hulse's (13) calculated amino acid scores for chick-pea and pigeon pea based upon (*a*) the FAO average values and (*b*) Hanuamantha Rao and Subramanian's analyses.

The amino acid score is the quotient of the amount of each amino acid reportedly present, divided by the WHO reference pattern level for the same amino acid. The first and second

Table 7. Composition of various fractions of chick-pea.

	Seed coat	Cotyledon	Embryo	Whole seed
Proportion (%)	14.5	84	1.5	100
Protein (N × 6.25) (%)	3	25	37	22
Ether extract (%)	0.2	5	13	4.5
Ash (%)	2.8	2.6	5	2.7
Crude fibre (%)	48	2	3	8
Carbohydrate (%)	46	66	42	63
Phosphorus (mg/100 g)	24	290	740	260
Iron (mg/100 g)	8	5	11	6
Calcium (mg/100 g)	1000	70	110	200

Table 8. Amino acid composition of pigeon pea (mg/g protein).

	a	b	c	d	e	f	g	h	WHO[i]
Isoleucine	51–66	57	66	38	30–33	31	50	46	40
Leucine	61–87	70	88	72	60–66	63	59	80	70
Lysine	62–74	64	70	68	72–83	77	59	58	55
Methionine	3–34	9	9	12	4–6	5	14	7)	
								)	35
Cystine	4–18	8	—	—	7–12	10	11	6)	
Phenylalanine	78–91	91	82	10	78–93	83	57	72)	
								)	60
Tyrosine	33–40	—	—	31	19–21	20	32	22)	
Threonine	31–40	38	41	36	28–31	29	47	40	40
Tryptophan	2–9	2	2	—	4–8	6	3	—	10
Valine	43–57	51	57	45	34–40	36	59	54	50
Histidine	—	34	22	34	35–40	37	—	36	—

[a]Hanuamantha Rao and Subramanian (10) (15 papers).
[b]Rao et al. (39).
[c]Banerjee (40).
[d]Van Etten et al. (41).
[e]FAO (4) — range.
[f]FAO (4) — average.
[g]Hanuamantha Rao and Subramanian (10) by paper chromatography.
[h]Royes (1972).
[i]WHO (1973).

Table 9. Amino acid composition of chick-pea (mg/g protein.)

	a	b	c	d	e	f	g	WHO[h]
Isoleucine	44–60	60	57	44	42–47	44	50	40
Leucine	49–80	86	67	76	71–80	75	50	70
Lysine	45–79	64	54	72	65–74	68	46	55
Methionine	7–31	17	9	14	5–17	10	9)	
							)	35
Cystine	7–18	8	—	—	8–15	12	8)	
Phenylalanine	30–68	50	37	66	39–78	57	53)	
							)	60
Tyrosine	20–35	—	—	33	19–34	29	23)	
Threonine	28–48	48	32	35	35–42	38	45	40
Tryptophan	2–12	6	4	—	4–15	9	3	10
Valine	38–63	54	45	46	34–57	45	48	50
Histidine	—	23	14	23	24–30	26	—	—

[a]Hanuamantha Rao and Subramanian (10) (15 papers).
[b]Rao et al. (39).
[c]Banerjee (40).
[d]Van Etten et al. (41).
[e]FAO (4) — range.
[f]FAO (4) — average.
[g]Hanuamantha Rao and Subramanian (10) by paper chromatography.
[h]WHO (1973).

Table 10. Amino acid scores.

	Chick-pea		Pigeon pea	
	a	b	a	b
Isoleucine	110	125	78	125
Leucine	107	71	90	84
Lysine	123	84	140	107
Methionine and cystine	63	49	43	71
Phenylalanine and tyrosine	143	126	172	148
Threonine	95	112	73	118
Tryptophan	90	30	60	30
Valine	90	96	72	118

[a]FAO — average.
[b]Hanuamantha Rao and Subramanian (10).

Table 11. Methionine content of chick-pea and pigeon pea.

Source[a]	No. samples	Mean methionine, mg/g sample	SD[b]	Range
Chick-pea	84	2.08	0.334	1.10–3.00
Pigeon pea	295	1.54	0.334	0.80–3.00

[a]Source: see ref. 8.
[b]SD, standard deviation.

Table 12. Biological efficiency of chick-pea and pigeon pea.

Source[a]	Biological value, %	Coefficient of digestibility, %	Protein efficiency	Level of feeding, %
Chick-pea	52–78	76–92	1.3–2.1	12
Pigeon pea	47–74	59–90	1.3–1.6	12

[a]Source: see ref. 25.

limiting amino acids are those with the lowest and second lowest score, respectively. According to the FAO results, the sulfur amino acids, methionine plus cystine, are first limiting in both chick-pea and pigeon pea. Tryptophan or valine are the second limiting in the case of chick-pea, and tryptophan is second limiting in pigeon pea. From Hanuamantha Rao and Subramanian's results, tryptophan appears clearly as first limiting and methionine plus cystine as second limiting in both cases. Braham et al. (14) claim that in autoclaved pigeon pea meal "methionine and tryptophan were equally deficient."

In IARI 1971 (10, p. 66) the results of methionine analyses on a large number of pulse crop samples are quoted. The data for chick-pea and pigeon pea are given in Table 11. Unfortunately, the results are quoted as milligrams methionine per gram of sample and hence cannot be compared with the amino acid results presented in other tables.

To what extent the variation in methionine is influenced by variability in protein content is not indicated. In any event, the range of results suggests that variability in methionine content may exist in a significant degree and may be genetically influenced.

The total lipid (ether extract) content of chick-pea appears in general to lie between 3 and 6% and in pigeon pea between 1 and 2%. The fatty acid composition of both legume lipids is nutritionally favourable with more than 50% of the lipid consisting of polyunsaturated fatty acids.

The carbohydrate content, which consists mainly of starch, is variously reported between 50

and 65% in both legumes.

In common with most other legumes, chick-pea and pigeon pea contain only modest amounts of vitamin A, approximately 300 IU/100 g (IU, International Units) in chick-pea and 150 IU in pigeon pea. Thiamine content in both legumes is approximately 0.5 mg/100 g. Both contain comparatively little riboflavin (approximately 0.15 mg/100 g) but both are fair sources of niacin (1.5–2.5 mg/100 g). All three vitamins are present in roughly the amounts found in whole cereal grains. Both chick-pea and pigeon pea are comparatively good sources of iron (6–9 mg/100 g) and contain 5–10 times the concentration of calcium found in the major cereals (Daniel and Norris (15) and Aykroyd and Doughty (16)).

Because in a number of countries chick-pea and pigeon pea are allowed to germinate before being eaten, a number of authors have reported on the influence of sprouting on a number of essential nutrients (De and Barai (17), Bannerjee and Bannerjee (18), Chattopadhyay and Bannerjee (19), De and Datta (20), Chattopadhyay and Bannerjee (21), Belavady and Bannerjee (22), Chattopadhyay and Bannerjee (23), Bannerjee et al. (24), Bannerjee et al. (25), and Singh and Bannerjee (26)). Ascorbic acid, niacin, available iron, choline, tocopherol, pantothenic acid, biotin, pyridoxine, inositol, and vitamin K all reportedly increase in both chick-pea and pigeon pea during germination.

Patwardhan (27) states that the biological value (BV) (an estimation of the proportion of absorbed nitrogen that is retained in the body for maintenance and (or) growth) ranges in chick-pea from 52 to 78% and in pigeon pea from 47 to 74%; that the coefficient of digestibility ranges from 76 to 92% in chick-pea and 59 to 90% in pigeon pea; that the protein efficiency ratio (PER) ranges from 1.3 to 2.1 in chick-pea and from 1.3 to 1.6 in pigeon pea (see Table 12). Elsewhere Patwardhan (28) quotes a PER of 1.1 for chick-pea and 0.7 for pigeon pea.

The variance cited by Patwardhan (27) is illustrated in other results from various authors. It is probable that these variable results reflect a combination of (*a*) differences in methodology, (*b*) intrinsic differences, and (*c*) differences in methods of processing the various samples reported on.

The comparatively low values of the various PERs quoted reflect the lack of balance in the amino acid content of these legumes. At the same time, in rat-feeding studies used to evaluate nutritional value, the results tend to be based upon isonitrogenous rather than isocaloric diets.

Some authors claim that cooking or autoclaving raises the nutritive value of both chick-pea and pigeon pea (Gaitonde and Sohoniek (29) and Hirwe and Magar (30)). Braham et al. (14) claim that after autoclaving for 20 min the PER of chick-pea meal was increased from 0.46 to 1.52.

Kande (31) states that normal cooking does not alter either the digestibility or the nutritive value of chick-pea.

Chitre and Vallury (32) compared the plasma protein levels of rats fed both raw and autoclaved chick-pea and pigeon pea. There was no significant difference between raw and autoclaved chick-pea but the plasma protein levels were lower in rats fed autoclaved pigeon peas than in rats fed raw pigeon peas. They concluded that chick-pea was one of the most efficient sources of protein in maintaining blood protein plasma levels.

Ochse (33) claims that raw seeds of pigeon pea contain an unidentified narcotic that if eaten in quantity induces sleepiness, and concludes that pigeon pea seeds are a harmless soporific. No one else to the author's knowledge has pursued this subject.

Kuppuswamy and Scrinivasan (34) report findings in Central America that indicate that chick-pea when fed as the sole source of protein

Table 13. Hemagglutinating and antitryptic activities of crude extracts[a] of raw legumes.

Legume	Hemagglutinating activity, (HU[b]/ml)	Antitryptic activity, (TIU/ml)
Phaseolus vulgaris		
Black bean	2450	2050
Kidney bean	3560	1552
Cicer arietinum	0	220
Cajanus cajan	0	418
Phaseolus aureus	0	260

[a]A 10% suspension of the finely ground meal in 1% NaCl clarified by centrifugation.
[b]HU, hemolytic unit.
[c]TIU, tesla international unit.

Table 14. Effect of heat on nutritive value of some legumes.

Source of protein	Gain in wt, g/day	
	Raw[a]	Heated
Phaseolus vulgaris		
Black bean	−1.94 (4–5)	+1.61
Kidney bean	−1.04 (11–13)	+1.48
Cicer arietinum		
Bengal gram	+1.25	+1.16
Cajanus cajan		
Red gram	+1.33	+1.74

[a]100% mortality observed during period (in days) shown in parentheses.

to experimental animals produced toxic symptoms attributable to "cicerism." It is claimed that the "toxicity" could be ameliorated by the addition of methionine or choline. No toxin was, however, identified and interest in "cicerism" seems to have died since 1951.

Three other undesirable characteristics associated with some food legumes are (*a*) the presence of substances that agglutinate red blood cells; (*b*) trypsin inhibitors; and (*c*) a tendency to induce flatulence. The first two factors have been studied by Liener (35), and his results are given in Tables 13 and 14. Hemagglutinating activity appears to be zero in chick-pea and pigeon pea and, compared with black bean and kidney bean, the antitryptic value in chick-pea and pigeon pea appears to be of little consequence.

Most of the comparative work on trypsin inhibitors has been done with bovine trypsin, which is more significantly affected by antitrypsins than is human trypsin; and because most of the trypsin inhibitors present in legumes appear to be comparatively thermolabile, it is doubtful if they are of any great importance in human diets. Certainly they appear to be of little consequence in cooked chick-pea and pigeon pea.

Though in adults flatus production is probably more of social than clinical importance, severe flatulence can give rise to acute discomfort in infants. Rao et al. (36) showed that chick-pea induced the highest amount of flatus, with black gram (*Phaseolus mungo*) next, followed by pigeon pea, and finally green gram (*Phaseolus radiatus*) having the lowest amount. Though the substance(s) in chick-pea and other legumes that leads to flatus has not been positively identified, it does appear that the effect is reduced by cooking. Srikantia (37) describes experiments in which groups of children received 50% of their total protein from pigeon pea, while another group received the same amount from milk. The growth of the children in the two groups was identical, suggesting that the legume protein was a satisfactory replacement for milk. The author states that "legumes could be used safely in amounts to provide as much as 50–60% of the total protein in the diet (of children)."

Hulse and Laing (13) and Urie and Hulse (38) have reported on the importance of phytic acid in human nutrition, which depends on its property of forming insoluble compounds with essential minerals such as calcium, iron, magnesium and zinc. Phytic phosphorus appears to be present in chick-pea at levels in excess of 200 mg/100 g. It is also present in significant levels in pigeon pea. The level in chick-pea approximates that present in whole wheat. Because the calcium content of chick-pea and pigeon pea is significantly higher than the calcium in cereals, the phytic phosphorus may not seriously interfere with calcium absorption in human diets. The phytin levels may, however, be sufficiently high to interfere with iron, magnesium, and zinc absorption.

Though the polyphenols (often described as "tannins") are known to be widely distributed among the Leguminosae, little appears to be known about the polyphenol content of chick-pea or pigeon pea. It seems highly probable, however, that polyphenols are present particularly in chick-pea possessing near-black, purple, brown or maroon seed coats and chick-peas with brown and orange testas. The biochemical mechanism whereby polyphenols interfere with protein metabolism in humans and animals has yet to be determined, but there is evidence to suggest that polyphenols can be correctly described as antinutrients. It would be worth discovering whether there is a significant difference in biological value between the dark- and light-seed coated pigeon pea and chick-pea varieties.

Hulse and Laing (13) have commented on the shortcomings of the manner in which analytical results related to the cereal grains are reported in the literature, and the need for a universally

standardized methodology by which the biochemical composition and the biological value of the cereal grains are determined and rationally presented.

Similar criticisms might be advanced concerning the published analytical and nutritional data relevant to the food legumes. The Protein Calorie Advisory Group (PAG) of the United Nations System has recently published, in PAG Guideline 16, *Protein Methods for Cereal Breeders Related to Human Nutritional Requirements* (39). Although many of the recommendations in this publication are applicable to legumes, it is hoped that in the not too distant future a similar PAG Guideline will be prepared for legume breeders. Some of the inherent difficulties and approaches to the subject are discussed in another PAG publication (4).

It is possible that chemical analysis is a less precise science than plant breeding. Williams (40) lists 27 sources of error in the Kjeldahl testing procedure for protein content and 18 sources of error in the Udy dye-binding system of protein testing. A significant error can result from dye-binding analyses carried out on immature grains, because the dye stuffs used are readily absorbed by chlorophyll and thus immature grains tend to give an exaggerated high value for protein content. In addition, grains high in cellulose may also present exaggerated high protein values. One of the greatest sources of error in amino acid analysis results from a lack of care and careful standardization of the method of hydrolysis.

Daniels (private communication 1974) carried out analysis of variance on the protein contents of various chick-pea and pigeon pea samples analyzed by micro-Kjeldahl and Udy (dye-binding) methods at ICRISAT, and the results are given in Table 15.

Though significant, the coefficients of correlation are comparatively low. This, at least in part, may be attributable to the narrow range of results over which the analyses were made. Because Udy is intended as a comparatively rough screening test, it would be useful to repeat the comparison over a much wider protein range. In any event, it is suggested that in selecting for increased protein, differences of less than 1 full percent in percentage protein (0.16% N) between the test and the standard can be discarded for all practical purposes.

As suggested in PAG Guideline 16 (39), it is urged that all analytical laboratories in plant improvement research centres establish collaborative protein and amino acid testing programs with other laboratories, and retain homogeneous reference samples stored below freezing in sealed containers to check equipment calibrations from time to time.

The biological methods of protein evaluation include those that depend on body weight gain and those that depend on nitrogen retention in the test animals. Most recommended test methods are based upon an isonitrogenous diet. It is readily demonstrable that the results with rats and other animals may be highly dependent upon the proportion of protein in the test diet. For example, proteins generally considered nutritionally inferior, such as wheat gluten, will appear more satisfactory at low levels of intake than at high levels of intake when compared with a standard protein such as casein. The PAG Guideline 16 therefore recommends a slope growth method in which all proteins are tested at at least three different levels against a standard, the rat being recommended as the preferable test animal. The relative protein value (RPV) is then expressed as: slope of the test protein/slope of the standard protein × 100.

A brief word on the standardization of conventions by which results are recorded is perhaps in order. It is recommended that "protein" values be quoted as total nitrogen on a dry weight basis. If it is considered desirable to quote the results as "protein" these results should

Table 15. Analysis of variation on protein analyses by micro-Kjeldahl and Udy.

	No. of samples	Range (protein %)	SD[a]	Coefficient of correlation
Chick-pea (whole)	29	21.5–25.13(MK) 22.58–26.56(Udy)	0.88 0.89	0.6171
Pigeon pea (whole)	85	18.1–23.31(MK) 19.26–23.17(Udy)	1.006 0.78	0.4152
Pigeon pea (seed coats removed)	14	23.52–27.58(MK) 23.64–26.24(Udy)	1.23 0.94	0.7912

[a]SD, standard deviation.

also be expressed on a dry weight basis and the conversion factor from "nitrogen" to "protein" clearly stated.

In the case of cereals it is recommended to breeders looking for "high protein" lines that they express their results as milligrams nitrogen per seed rather than as nitrogen or protein on a total dry weight basis. In the cereal grains, protein content and composition vary among different fractions of the seed and protein, as percentage dry matter is influenced by seed weight and the relative proportion of the various seed fractions present. These in turn are influenced by environment and agronomic conditions. Similarly, the protein–nitrogen present in the legumes is not uniformly distributed throughout the seed (11) and therefore results expressed as milligrams nitrogen per seed is again recommended when selecting for higher protein breeding lines.

Amino acids have also been expressed in a variety of ways. It is recommended that no matter what the method of determination the results should be expressed as milligrams of amino acids per gram nitrogen. Minerals and vitamins are best expressed as milligrams or grams per 100 g of material with the exception of vitamins A and D, which are customarily expressed in International Units.

It would appear that if resources are to be used with greatest effect a great deal more cooperation between plant breeders on the one hand and analytical chemists and nutritional biochemists on the other hand is essential. It is my view that the latter have served the breeders very poorly in their attempts to develop plants of superior nutritional values. Perhaps an elementary course in botany would prove valuable for food chemists and biochemists.

In seeking genotypes capable of synthesizing higher than average levels of protein nitrogen some attention might be given to the duration of nitrogenase activity. Hardy et al. (41) and LaRue and Kurz (42) have described a method for determining the duration of nitrogenase activity, which depends on the ability of the nitrogenase present in the legume root nodule to reduce acetylene to ethylene. The results of the workers at the Prairie Regional Laboratory in Saskatoon indicate significant variations among different *Pisum sativum* lines in the length of time during which the nitrogenase is active. It is their belief that those lines of longer nitrogenase activity possess a higher potential capacity for synthesizing seed protein.

A PAG Working Group (4) recommends a long list of considerations to which the plant breeder should give attention. Time will not permit a detailed commentary on the individual recommendations within the PAG document but suffice it to say that, because pigeon pea and chick-pea appear to be comparatively free from major toxic factors and nutritional inhibitors, the plant breeder's primary concern should be to increase the yield potential of these crops, and to explore the range of genetic variability related to seed nitrogen content and perhaps amino acid composition. What is required is a significant increase in protein production per unit area of land per unit of time. As a secondary objective, and when time and facilities permit, it would be useful to determine whether the proportions of sulfur-containing amino acids and tryptophan are genetically controlled. If either of these limiting amino acids is increased it should not be at the expense of lysine. The cereal grains are first limiting in lysine and because cereals and legumes are eaten together in many diets, the lysine contribution by the legume is of primary importance. Where the legumes are eaten with maize there may be a good case for attempting to raise the tryptophan content, though this may be more readily achieved by genetic manipulation of the maize than of the legumes.

Where chick-pea and pigeon pea are the principal sources of protein nitrogen calories in the diets of people who subsist largely on root crops, the sulfur amino acid content is of significant importance, because the cyanogenic glycosides present in cassava combine with and reduce the absorption of methionine and other sulfur amino acids.

It is my opinion that a great deal more attention could be given to the processing of legumes. As stated earlier, food legumes tend to be regarded as poor man's meat but this image could be significantly changed by imaginative technological research and development. Particularly to be recommended are technological systems that permit the processing of cereals and legumes using the same equipment. Such technology has been developed in Canada and is now installed in a small rural mill in Northern Nigeria. It consists of simple abrasive decortication using rotary Carborundum discs in a rubber case followed by hammer mills or mosaic grinders, screening, and packaging facilities. Technology of this kind permits the production of inexpensive foods in which the optimum ratio of cereal and legume protein is combined. Such foods are particularly advantageous for infants and young children, nursing mothers, and other nutritionally vulnerable groups.

The techniques of protein concentration in

cereals by fine grinding and air classification have been known for many years. The principle is that in finely ground flour the carbohydrate preponderates in the heavier particles. Consequently, protein fractionation can take place by applying a centrifugal force to the fine flour particles opposed by a centripetal drag. The heavier particles of higher effective mass will move in one direction and the finer protein rich particles in the other. In theory, air classification is easier to achieve with legume flours than with cereal flours because legume flours contain, in general, significantly larger starch granules. That this theory is sound has been demonstrated at the Prairie Regional Laboratories in Canada (private communication) where field pea flour (*P. sativum*) has been thus converted to significantly higher protein contents than occur naturally in the cotyledon.

Although this technology is not as inexpensive as simple milling, it is simpler, less expensive, and less hazardous in tropical countries to operate than the "wet" systems of producing protein concentrates.

In summary, it can be said that the chick-pea particularly, and also the pigeon pea, represent valuable but considerably underexploited sources of edible protein. Greater attention needs to be given to their genetic diversity to determine the range of variability related to their biochemical composition. However, as stated at the outset, breeding for improved nutritional quality should not be undertaken at the expense of all those factors that contribute to improved yield.

References

1. Arnon, I. 1972. Crop production in dry regions, vol. 2. London, England, Hill (Leonard) Books, 683.
2. Watt, Sir G. 1908 (reprinted 1966). The commercial products of India. New Delhi, India, Today & Tomorrow's Printer and Publisher.
3. De Candolle, A. 1967 (reprint of 1886 ed.). The origin of cultivated plants, 2nd ed. New York, N.Y., U.S., Hafner Publishing Company Inc.
4. Swaminathan, M.S., and Jain, H.K. 1972. Nutritional improvement of food legumes by breeding. New York, U.S., United Nations Protein Advisory Group.
5. Food and Agricultural Organization of the United Nations. 1972. FAO Production Yearbook.
6. Food and Agricultural Organization of the United Nations. 1970. FAO Amino acid content of foods.
7. Argikar, G.P. 1970. Pulse crops of India. New Delhi, India, Indian Council for Agricultural Research.
8. Lal, B.M., Prakash, V., and Verma, S.C. 1963. The distribution of nutrients in the seed parts of Bengal gram (chick-pea). Experientia (Vicosa Minas Gerais, Brazil), 19(3), 154–155.
9. Chandra, S., and Arora, S.K. 1968. Curr. Sci. (Bangalore), 37, 237.
10. Indian Agricultural Research Institute. 1971. New vistas in pulse production.
11. Zimmerman, G., Weismann, S., and Yannai, S. 1967. The distribution of protein, lysine and methionine, and antitryptic activity in the cotyledons of some leguminous seeds. J. Food Sci. (U.S.), 32, 129–130.
12. Hanumantha, K. Rao, and Subramanian, N. 1970. Essential amino acid composition of commonly used Indian pulses by paper chromatography. J. Food Sci. Technol. (India), 7, 31–34.
13. Hulse, J.H., and Laing, E.M. 1974. Nutritive value of triticale protein. Ottawa, International Development Research Centre, IDRC-021e.
14. Braham, J.E., Elías, L.G., and Bressani, R. 1965. Factors affecting the nutritional quality of cottonseed meals. J. Food Sci. (U.S.), 30, 531–537.
15. Daniel, L., and Norris, L.C. 1945. The riboflavin, niacin and thiamine content of dried leguminous seeds. J. Nutr. (U.S.), 30, 31–36.
16. Aykroyd, W.R., and Doughty, J. 1964. Legumes in human nutrition. FAO Nutritional Study No. 19. Rome, Italy, Food and Agriculture Organization of the United Nations.
17. De, H.N., and Barai, S.C. 1949. Study of the mechanism of biosynthesis of ascorbic acid during germination. Indian J. Med. Res. (India), 37, 101–110.
18. Bannerjee, S., and Bannerjee, R. 1950. Indian J. Med. Res. (India), 38, 153.
19. Chattopadhyay, H., and Bannerjee, S. 1951. Studies on the choline content of some common Indian pulses and cereals both before and during the course of germination. J. Food Sci. (U.S.), 16, 230–232.
20. De, H.N., and Datta, S.C. 1951. Studies on the mechanism of biosynthesis of nicotinic acid during germination of cereals and pulses. Indian J. Agric. Sci. (India), 31(4), 16.
21. Chattopadhyay, H., and Bannerjee, S. 1952. Effect of germination on the total tocopherol content on pulses and cereals. J. Food Sci. (U.S.), 17, 402–403.
22. Belavady, S., and Bannerjee, S. 1953. Studies on the effect of germination on the phosphorus values of some common Indian pulses. Food Res. (U.S.), 18, 223.
23. Chattopadhyay, H., Bannerjee, S. 1953. Effect of germination on the biological value of proteins and the trypsin-inhibitor activity of some common Indian pulses. Indian J. Med. Res. (India), 41, 185–189.
24. Bannerjee, S., Rohatgi, K., and Lahin, S. 1954. Food Res. (U.S.), 19, 134.
25. Bannerjee, S., Rohatgi, K., Bannerjee, M., Chattopadhyay, O., and Chattopadhyay, H. 1955. Food Res. (U.S.), 20, 545.
26. Singh, H.D., and Bannerjee, S. 1955. Studies on the effect of germination on the availability of iron in some common Indian pulses. Indian J. Med. Res. (India), 43, 497–501.

27. Patwardhan, V.N. 1962. Pulses and beans in human nutrition. Am. J. Clin. Nutr. (U.S.), 2, 12.
28. Patwardhan, V.N. 1961. Nutritive value of cereal and pulse proteins. Washington, D.C., U.S., National Academy Journal Sciences, National Research Council, Publication 843.
29. Gaitonde, M.K., and Sohoniek, J. 1952. On the occurrence of a trypsin inhibitor in field bean and a few other common Indian pulses. J. Sci. Ind. Res. (India), 11B, 339–341.
30. Hirwe, S.N., and Magar, G. 1951. Effect of autoclaving on the nutritive value of Bengal gram, Dhal, Arhar and Lentil. Curr. Sci. (Bangalore), 20, 40–41.
31. Kande, J. 1967. Valeur nutritionelle de deux graines de lentille (*Lens esculenta*). Ann. Nutr. Aliment (France), 21, 45–67.
32. Chitre, R.G., and Vallury, S.M. 1956. Studies on the protein value of cereals and pulses, Part II. Indian J. Med. Res. (India), 44, 565–571.
33. Ochse, J.J. 1931. Vegetables of the Dutch East Indies. The Hague, Netherlands, Martinus Nijhoff.
34. Kuppuswamy, S., and Scrinivasan, M. 1958. Proteins in foods. Indian Counc. Med. Res. Annu. Rep. (India), 26, 35–56.
35. Liener, I.E. 1973. Toxic factors associated with legume proteins. J. Nutr. Diet. (India), 10, 303.
36. Rao, M.N., Shurpalekar, K.S., Sundaravalli, E.E., and Doraiswamy, T.R. 1973. Flatus production in children fed legume diets. Protein Advisory Group Bulletin, 3(2), 53.
37. Srikantia, S.G. 1973. Use of legumes and green leafy vegetables in the feeding of children: a review of experience. Protein Advisory Group Bulletin, 3(2), 30.
38. Urie, A., and Hulse, J.H. 1952. The science, raw materials and hygiene of baking. London, England, MacDonald & Evans Ltd.
39. Protein-Calorie Advisory Group of the United Nations. 1975. PAG Guideline (No. 16) on protein methods for cereal breeders as related to human nutritional requirements. PAG Bull. V(2), 22–48.
40. Williams, P.C. 1974. Errors in protein testing. Cereal Sci. Today (U.S.), 19(7), 281–286.
41. Hardy, R.W.F., Holsten, R.D., Jackson, E.K., and Burns, R.C. 1968. The acetylene–ethylene assay for N_2 fixation: laboratory and field evaluation. Plant Physiol. (Bethesda, U.S.), 43, 1185–1207.
42. Larue, I.A.G., and Kurz, W.G.W. 1972. Estimation of nitrogenase in intact legumes. Can. J. Microbiol. (Canada), 19, 304–305.
43. Rao, P.V., Ananthachar, T.K., and Desikachar, H.S.R. 1964. Effect of certain chemicals & pressure on cookability of pulses. 417–418.
44. Bannerjee, S. 1960. Biological value and essential amino acid composition of the proteins and some pulses. 355–356.
45. Van Etten, C.H., Kwolek, W.F., Peters, J.E., and Barclay, A.S. 1967. Plant seeds as protein sources of food or feed. Evaluation based on amino acid composition of 379 species. J. Agric. Food Chem. (U.S.), 15, 1077–1089.

Additional References Not Indicated in Text

Acharya, B.N., Niyogi, S.P., and Patwardhan, V.N. 1942. The effect of parching on the biological value of the proteins of some cereals and pulses. Indian J. Med. Res. (India), 30, 73–81.

Adolph, W.H., Shammas, E.I., and Halaby, S.H. 1955. The nutritive value of legume proteins and legume-wheat mixed proteins in Near East diets. J. Am. Diet. Assoc. (U.S.), 20, 31–34.

Ashur, S.S., Clark, H.E., Moon, W.H., and Malzer, J.L. 1973. Nitrogen retention of adult human subjects who consumed wheat and rice supplemented with chickpea, sesame, milk, or whey. Am. J. Clin. Nutr. (U.S.), 26, 1195–1201.

Barja, I., Munoz, P., Solimano, G., Vallejos, E., Undurraga, O., and Tagle, M.A. 1971. Formula de garbanzo (*Cicer arietinum*) en la alimentacion del lactante sano. Arch. Latinam. Nutr. (Venezuela), 21(4), 486–492.

Bhagvat, K., and Narasinga Rao, K.K.P. 1942. Vitamin C content of dry Bengal gram (*Cicer arientinum*). Indian J. Med. Res. (India), 30(4), 505–511.

Butt, J.M., Hamid, A., and Shah, F.H. 1965. Changes in Vitamin 'C' contents of germinating seeds. Black Bengal grams (*Cicer arietinum*) Part 1. Pak. J. Sci. Res. (Pakistan), 17(3–4), 164–168.

Chitre, R.G., and Vallury, S.M. 1956. Studies on the protein value of cereals and pulses, Part 1. Indian J. Med. Res. (India), 44, 555–563.

Daniel, V.A., Desai, B.L.M., Subrahmanya Raj Urs, T.S., Venkat Rao, S., Swaminathan, M., and Parpia, H.A.B. (with statistical analysis by Rajalakshmi, D.) 1968. The supplementary value of bengal gram, red gram, soya bean as compared with skim milk powder to poor Indian diets based on ragi, kaffir corn and pearl millet. J. Nutr. Diet. (India), 5(4), 283–291.

Daniel, V.A., Desai, B.L.M., Venkat Rao, S., Swaminathan, M., and Parpia, H.A.B. 1970. The effect of supplementing with limiting amino acids on the nutritive value of the proteins of low cost balanced foods based on blends of cottonseed, peanut and chickpea flours and kaffircorn (*Sorghum vulgare*) or ragi (*Eleusine coracana*) or wheat. Plant Foods Hum. Nutr. (U.S.), 2, 1–6.

Daniel, V.A., Narayanaswamy, D., Desai, B.L.M., Kurien, S., Swaminathan, M., and Parpia, H.A.B. (with statistical analysis of results by Rajalakshmi, D.) 1970. Supplementary value of varying levels of red gram (*Cajanus cajan*) to poor diets based on rice and ragi. J. Nutr. Diet. (India), 7, 358–362.

Daniel, V.A., Subramanya Raj Urs, T.S., Desai, B.L.M., Venkat Rao, S., Rajalakshmi, D., Swaminathan, M., and Parpia, H.A.B. 1967 & 1968. Studies

on low cost balanced foods suitable for feeding weaned infants in developing countries. The protein efficiency ratio of low cost balanced foods based on ragi or maize, groundnut, Bengal gram, soya and sesame flours and fortified with limiting amino acids. J. Nutr. Diet. (India), 4, 183–291.

De, H.N., and Datta, S.C. 1952. Studies on the mechanism of biosynthesis of nicotinic acid during germination of cereals and pulses. Indian J. Agric. Sci. (India), 21, 375–394.

Easwaran, P.P., Gopinath, S., Jamala, S., and Devadas, R.P. 1972. Evaluation of the protein quality of two selected vegetable protein mixtures using albino rats. J. Nutr. Diet. (India), 9, 327–330.

Esh, G.C. and De, T.S. 1960. High and low protein Bengal gram: nitrogen distribution and nutritional assessment. 1, 357–362.

Goyco, J.A., and Asenjo, C.F. 1965. The lactation value, a new index of protein evaluation. J. Nutr. (U.S.), 85, 52–56.

Grande, F., Anderson, J.T., and Keys, A. 1965. Effect of carbohydrates of leguminous seeds, wheat and potatoes on serum cholesterol concentration in man. J. Nutr. (U.S.), 86, 313–317.

Hallab, A.H., Khatchadourian, H.A., and Jabr, I. 1974. The nutritive value and organoleptic properties of white Arabic bread supplemented with soybean and chickpea. Cereal Chem. (U.S.), 51(1), 106–112.

Hanafy, M.M., Seddik, Y., and Aref, M.K. 1970. Formulation of a protein-rich vegetable mixture for prevention of protein-calorie malnutrition. J. Food Agric. (England), 21(1), 8–18.

Holl, F.B., and LaRue, T.A. 1967. Genetics of legume plant hosts. J. Food Sci. (U.S.), 32, 1–17.

Kurien, P.P., and Parpia, H.A.B. 1968. Pulse milling in India — I processing and milling of Tur, Arhar (*Cajanus cajan* Linn). 203–207.

Kurien, S., Narayanaswamy, D., Daniel, V.A., Swaminathan, M., and Parpia, H.A.B. 1971. Supplementary value of pigeon pea (*Cajanus cajan*) and chickpea to poor diets based on kaffir corn and wheat. 4(4), 229–236.

Mathur, K.S., Singhal, S.S., and Sharma, R.D. 1964. Effect of Bengal gram on experimentally induced high levels of cholesterol in tissues and serum in albino rats. J. Nutr. (U.S.), 84, 201–204.

Mathur, K.S., Wahi, P.N., Gahlaut, D.S., Sharma, R.D., and Srivastava, S.K. 1961. Prevalence of coronary heart disease in general population at Agra. Indian J. Med. Res. (India), 49, 605–611.

Narayanaswamy, D., Doraiswamy, T.R., Daniel, V.A., Swaminathan, M., and Parpia, H.A.B. 1972. Effect of supplementing poor rice diet with low cost protein food, chick pea or skim milk powder on nitrogen retention and net protein utilisation in children. Nutr. Rep. Int. (U.S.), 5(2), 171–181.

Parpia, H.A.B. 1972. Utilization problems in food legumes. 281–295.

Phansalkar, S.V., Ramachandran, M., and Patwardhan, V.N. 1957. Nutritive value of vegetable proteins (Part 1). Indian J. Med. Res. (India), 45, 610–621.

Ramakrishna, M., Wankhede, D.B., and Raghavendra Rao, M.R. 1973. Incorporation of (U-14C) aspartate into lysine, threonine and other amino acids in Bengal gram (*Cicer arietinum*). Indian J. Biochem. (India), 10(4), 285–286.

Rao, K.H., and Subramanian, N. 1970. Essential amino acid composition of commonly used Indian pulses by paper chromatography. J. Food Sci. Technol. (India), 7, 31–34.

Rao, N.N., Ramachandra Rao, T.N., and Shanthamma, M.S. 1972. Development of pre-digested protein rich food based on Indian oilseed meals and pulses II. J. Food Sci. Technol. (India), 9(2), 57–62.

Shehata, N.A., and Fryer, B.A. 1971. Effect on protein quality of supplementing wheat flour with chickpea flour. Cereal Chem. (U.S.), 47(6), 663–670.

Singh, L., Maheshwari, S.K., and Sharma, D. 1971. Effect of date of planting and plant population on growth, yield, yield components and protein content of pigeon-pea (*Cajanus cajan* (L.) Millsp.). Indian J. Agric. Sci. (India), 41(6), 535–538.

Singh, S., Singh, H.D., and Sikka, K.C. 1968. Distribution of nutrients in the anatomical parts of common Indian pulses. Cereal Chem. (U.S.), 45, 12–19.

Sinha, S.K. 1973. Green revolution and break-through in food production in India. Indian J. Agric. Econ. (India), 28(2), 26–42.

Srinivasa Rao, P. 1969. Studies on the digestibility of carbohydrates in pulses. Indian J. Med. Res. (India), 57, 2151–2157.

Tara, M.R., and Rama Rao, M.V. 1972. Changes in the essential amino acid content of Arhar Dhal (*Cajanus cajan*) on dehydration. 76–79.

Tara, M.R., Rawal, T.N., and Rama Rao, M.V. 1972. Effect of processing on the proteins of Arhar Dal. J. Nutr. Diet. (India), 9, 208–212.

Tasker, P.K., Narayanarao, M. Swaminathan, M., Sankaran, A.N., Jayaraj, A.P., and Subrahmanyan, V. 1961. The supplementary value of a low-cost protein food based on a blend of peanut, coconut, and chickpea (*Cicer arietinum*) flours to a maize-tapioca diet. J. Agric. Food Chem. (U.S.), 9(5), 413–416.

Tomar, G.S., Singh, L., Sharma, D., and Deodhar, A.D. 1973. Phenotypic stability of yield and some seed characteristics in Bengal gram (*Cicer arietinum* L.) varieties. JNKVV Rs. J. 7(1), 35–39.

Vankat Rao, S., Leela, R., Swaminathan, M., and Parpia, H.A.B. 1964. The nutritive value of the proteins of leguminous seeds. J. Nutr. Diet. (India), 1, 304–321.

Vijayaraghavan, P.K., and Srinivasan, P.R. 1953. Essential amino acid composition of some common Indian pulses. J. Nutr. (U.S.), 51, 261–271.